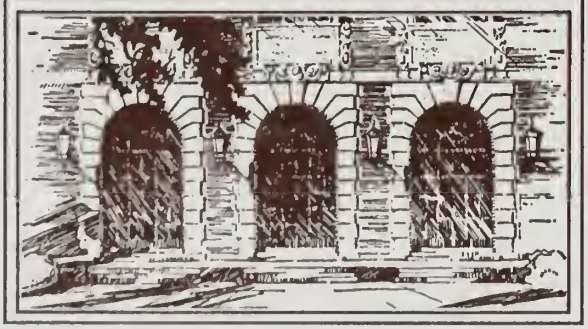

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VIRUS REMOVAL BY CHEMICAL COAGULATION

By
MALAY CHAUDHURI

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Removal of viruses by chemical coagulation was studied in the laboratory. Using bacterial viruses (bacteriophages T4 and MS2 against Escherichia coli) as models and aluminum as the coagulant metal ion, it was shown that removal of viruses from water by chemical coagulation with aluminum sulfate consists of a primary reaction step which involves an interaction between aluminum and the virus coat protein. The reaction was found to be instantaneous and proceeded according to a definite stoichiometry. Neither the kinetics nor the stoichiometry was affected by the period of storage of the virus particles, the quantity of available aluminum or the presence of bivalent cations like calcium and magnesium. Aluminum adsorption data were found to fit the Langmuir adsorption equation. Amounts of aluminum adsorbed by a single virus particle at pH values 5.0, 6.0 and 9.0 were calculated and found to be comparable. Considering the aqueous chemistry of aluminum, the amino acid composition of virus coat protein and the literature evidences on aluminum-protein interaction, it was concluded that the interaction between aluminum and virus possibly resulted in the formation of coordination complexes between aluminum and carboxyl groups on the virus coat protein. The complexed viruses were not inactivated and active viruses could be recovered from the settled floc following their removal from water by coagulation and flocculation.

The process of chemical coagulation and flocculation was found quite effective in removing bacteriophages T4 and MS2 from water. The optimum coagulant dosages and pH values were 40 to 50 mg/l of aluminum sulfate at

pH 5.24 for bacteriophage T4 and at pH 6.0 for bacteriophage MS2. The highest removals attained were 98.0 and 99.9 percent, respectively. Presence of bivalent cations like calcium and magnesium up to a concentration of 50 mg/l each did not interfere with the efficiency of the process. Organic matter like albumins, wastewater and wastewater effluent lowered the removal efficiency significantly. Commercially available cationic polyelectrolytes were found effective both as coagulant aids and as prime coagulants.

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I. INTRODUCTION

The need of processes for the removal or inactivation of water-borne viruses has been demonstrated beyond question by virus transmission in drinking water and polluted streams. Because of the decrease in the available water supplies, the potential health hazard of viral pollution is increasing, and more knowledge on the survival and removal of viruses in water and wastewater treatment processes is needed.

Viruses have been demonstrated to be the causative agents of a wide variety of diseases. Polio, Coxsackie, Infectious Hepatitis, ECHO, and Adenoviruses have all been demonstrated in the feces of infected humans (Clarke et al., 1964). Of greater significance is the fact that a great many of these viruses may be found in wastewater treatment plant effluents (Kelly and Sanderson, 1959). Enteric viruses have been found in raw wastewater and treatment plant effluents by a number of investigators (Paul, Trask and Gard, 1940; Paul and Trask, 1942; Clarke et al., 1951; Kelly, Winsser and Winkelstein, 1957; Mack et al., 1958; Carlson, Ridenour and McKhann 1943; and Bancroft, Engelhard and Evans, 1957). The presence of these organisms in water supplies, then, is certainly possible. Although water is far from being an ideal medium for sustaining viruses, they can exist in it for substantial periods of time. In Paris, France, more than six virus isolations have been made from tap water in at least four different sections of the city (Berg, 1964). Water has been suspected as being the mode of transmission in outbreaks of several virus diseases. At least four epidemics plus numerous lesser outbreaks of viral diseases have been attributed to water-borne viruses (Bancroft, Engelhard and Evans, 1957; Dennis, 1959; Hayward, 1946; Little, 1954; and Standly and Eliassen, 1960). Hudson (1962), in attempting to correlate

drinking water quality with the incidence of infectious hepatitis, found that a water supply that met all existing standards could still transmit these diseases. This would indicate that a specific virus quality control or standard should be established for drinking water supplies (Hartung, 1961). Before this can logically be accomplished, more knowledge should be gained on the effectiveness of presently used water and wastewater treatment methods for the removal of viruses and the epidemiology of water-borne viral diseases.

Although the process of chemical coagulation and flocculation has been used for many years in the treatment of water supplies, little is known of the basic physico-chemical principles involved in the removal of dead, as well as living, organic matter from the treated water. Chemical coagulation and flocculation is applied in various forms in a modern water treatment plant to produce a safe and potable water. However, little information is available about the basic mechanisms and kinetics involved in the removal of viruses by this unit process. Virus removals ranging from 25 to 99 percent have been reported by different workers (Senn et al., 1961). Understanding the mechanism by which viruses, which behave as typical proteins in water, are removed from water by the process of chemical coagulation and flocculation is important in optimizing its use.

The present work was undertaken in order to delineate the basic mechanisms involved in the removal of viruses by chemical coagulation. Studies were also conducted to investigate the quantitative and practical aspects of the process. Various parameters, believed to affect the process, were evaluated. It is believed that the information obtained from this investigation, though it may not be quantitatively applicable to a particular water treatment plant, will contribute fundamental knowledge regarding the removal of viruses from water by chemical coagulation and flocculation and the various environmental parameters affecting the process.

II. PRESENT STATE OF KNOWLEDGE

A. Viruses

(1) Physical and Chemical Nature

Viruses are the smallest known biological form capable of producing disease in humans and in other living species. They are obligate intracellular parasites, incapable of proliferating in an extracellular environment. Viruses are known to infect man, other vertebrates, plants, insects, and bacteria. Those infecting bacteria have been termed bacterial viruses (or bacteriophages), while those infecting man and other vertebrates are known as animal viruses. The Rickettsia, basophilic viruses, and pleuropneumonia-like organisms (PPLO) are usually considered, both physically and biologically, intermediates between the bacteria and viruses. General properties of viruses can be briefly outlined as follows:

a. Viruses have definite shapes and structures. Some appear to be spherical, others appear to be brick-shaped, or filamentous. Bacterial viruses may have tails, which give them a sperm-like appearance. In recent years, the surfaces of many viruses have been clearly photographed. These photographs have resulted in a clearer understanding of the viral capsid, the outer cell, which is composed of many subunits, or capsomers.

b. Viruses of the same type are characterized by a definite size but as a group they differ in size. Some, such as poliovirus, are on the order of 30 mμ or less in diameter while others may approach 400 mμ - a size approximating that of some bacteria.

c. Viruses are composed of known chemical substances, of which protein and nucleic acid are the most important. In solution, viruses behave

as proteins, though the internal structure may be nonprotein in character. In their electrokinetic properties, viruses behave as amphoteric electrolytes. The net charge depends on the pH of the solvent. Most viruses display a net negative charge within the range of pH stability.

d. Viruses breed true to form, meaning that the progeny (offspring) derived from a virus particle are like the parent virus. This phenomenon is controlled by the nucleic acid contained in a virus.

e. Viruses display specificity with regard to their protein and to the host cell which they infect.

f. Viruses can change the life processes of a cell, causing injury, a modification in growth rate, or even death. Some viruses infect a cell without causing any recognizable effect. In such an instance, the virus can remain dormant indefinitely or can be stimulated to multiply.

g. Some viruses are extremely sensitive to environmental conditions - temperature, ultraviolet light, etc., while others are fairly resistant to chemical and physical factors.

h. Some viruses are transmitted directly to their hosts; others require an intermediate host.

(2) Enteric Viruses and Diseases Associated With Them

Unfortunately, a binomial system of nomenclature as is commonly used to classify bacteria is not readily applicable to animal viruses. For a phylogenic classification there is not, as yet, sufficient knowledge of virus properties nor of the relationships viruses hold to one another. Furthermore, probably only a small fraction of all the animal viruses have yet been discovered (Rhodes and VanRooyen, 1962). Perhaps the most reasonable classifications of animal viruses are those which assign viruses with similar properties to groups designated by distinguishing names and the suffix "virus."

Under this system of nomenclature the viruses of our concern fall within four major groups: the adenovirus group, the reovirus group, the enterovirus group, and the hepatitis group. Collectively, these are referred to as "enteric viruses." Table 1 lists these viruses together with the diseases with which they have been associated. All enteric viruses share a number of common characteristics (Clarke and Chang, 1959):

a. They are excreted in feces in large numbers. Poliovirus, for example, has been reported in concentrations as high as 100,000 virus units per gram of feces.

b. They can be found in municipal wastewater, particularly during the late summer and early fall.

c. Infection with many of these viruses is widespread in the normal population; infection rates are highest in infants and young children.

d. Illness, when it occurs with infection with one of these viruses, may be so mild as to be mistaken for a slight cold or, in rare cases, may be as severe as paralytic poliomyelitis.

(3) Water-borne Viruses and the Hazard of Viruses in Water

Undisputably viruses exist in water (Gard, 1940, and Kelly, Winsser and Winkelstein, 1957). Many are introduced into surface waters and municipal sewer systems through human and animal feces. All enteric viruses occur in excreted feces in considerable numbers. Over 100 different viruses are excreted in human feces, including three types of Poliomyelitis, 30 types of Coxsackie, 28 types of ECHO, Infectious Hepatitis and various Adenoviruses (Weibel et al., 1964; Berg, 1964; and Rhodes and VanRooyen, 1962). The typical expected density of enteric viruses in municipal wastewater would average about 7000 per liter of raw, untreated wastewater (Clarke and Kabler, 1964), and in polluted surface water not more than one virus unit per 100 ml

TABLE 1

ENTERIC VIRUSES AND DISEASES WITH WHICH THEY HAVE BEEN CLOSELY ASSOCIATED
(After Berg, 1966)

Virus*	Disease								
	Paralytic Poliomyelitis	Aseptic Meningitis	Pleurodynia	Herpangina	Respiratory Illnesses	Enteritis	Rash Diseases	Acute Infantile Myocarditis	Jaundice
Polioviruses	X	X	-	-	-	-	-	-	-
Coxsackieviruses group A	-	X	-	X	-	-	-	-	-
Coxsackieviruses group B	-	X	X	-	-	-	-	X	-
Echoviruses	-	X	-	-	X	X	X	-	-
Adenoviruses	-	-	-	-	X	-	-	-	-
Reoviruses	-	-	-	-	X	X	-	-	-
Infectious hepatitis virus(es)	-	-	-	-	-	-	-	-	X

*Only certain strains within the designated groups have been proven to be responsible for the designated diseases.

(Clarke et al., 1964). These values, of course, would be subject to wide variations and could change radically during a virus disease epidemic.

In a recent review of experimental data, Plotkin and Katz (1966) concluded that one cell culture infective dose is sufficient to infect a human. This indicates that if viruses are isolated from water that is consumed by man, there is sufficient virus in that water to infect a proportion of those that consume the water. No one has determined what this proportion is, but in Paris, France, viruses are readily isolated from the tap water (Coin et al., 1965). According to Mosley (1966) there have been a total of 50 episodes of infectious hepatitis reported in the literature in which the investigators concluded that infectious hepatitis was transmitted by drinking water. Outbreaks of poliomyelitis have been suspected from wastewater-contaminated water supplies (Kabler et al., 1961; Little, 1954; and Bancroft, Engelhard and Evans, 1957). Poliomyelitis virus has been isolated from well water supplies in both the United States and Sweden and from a creek in Ohio (Clarke and Chang, 1959). Outbreaks of sore throats and pink eye with fever have occurred in Washington, D. C. and Toronto, Canada, from bathing in areas contaminated by Adenovirus types 3 and 7 (McLean, 1964, and Kabler et al., 1961). Enteroviruses in swimming pool thus constitute a problem. These two occurrences are not the only incidents associated with bathing areas. Enteroviruses were isolated from wading pools in Albany, New York (Kelly and Sanderson, 1961) and in Toronto, Canada (McLean, 1966). In 1957, spread of vesicular exanthema in Toronto, caused by Coxsackie A16 virus, was aided by backyard swimming pools (McLean, 1964).

It has been established that viruses are present in surface waters receiving wastewater effluents. There is, therefore, an urgent need for further investigation on the virus content of wastewater effluents, the relative

efficacy of different types of wastewater treatment, the consequent virus pollution of rivers, the survival of viruses in natural waters and the effectiveness of water treatment processes in their removal or inactivation.

(4) Survival of Viruses in Water

The rate of viral inactivation in natural waters depends upon temperature, water quality, predators, and other factors that are not well understood (Berg, Scarpino and Berman, 1966). Due to the existence of many unknown factors affecting virus survival in water, the differences in the survival of different enteroviruses observed in storage (Clarke et al., 1964) are not sufficient to indicate that some enteroviruses survive significantly longer than others. In general, a 99.9 percent reduction requires from a few weeks in the warm season to a few months in the cold season in temperate regions and a shorter time in the tropics and subtropics (Chang, 1968). Virus survival data are summarized in Table 2. It may be concluded that the storage time normally available to water treatment plants is not to be depended upon for any significant reduction in the enteric virus concentration.

B. Detection of Viruses in Water

Studies on the concentration of viruses in water and wastewater and the evaluation of water and wastewater treatment methods in removing viruses have been severely limited due to the lack of suitable quantitative methods for virus detection. Low densities of viruses in natural waters require that large volumes of water be collected and subsequently concentrated before virus detection and isolation. According to Chang (1968), a method suitable for detection of viruses in water will have to fulfill the following qualifications: (1) it can concentrate small numbers of viral units from large volumes of water, (2) the virus material thus concentrated can be conveniently inoculated into cell cultures for determination of viral density by the tissue

TABLE 2

SURVIVAL OF ENTERIC VIRUSES IN WATER

Viruses	Type of Water	Probable Storage Necessary for 99 Percent Reduction in Days (and temperature °C)	Survival Time in Days (and Temperature °C)	References
Polio	River Water with Feces		Present after 188 days	Rhodes et al. (1950)
	River Water	<15(15 - 16); < 7(20 - 23)	11(20)	Poynter (1968)
	Distilled Water	> 100(8 - 10)	91(20)	Gilcreas and Kelly (1955); Poynter (1968)
	Seawater in Laboratory		> 130(5); 28(37)	Metcalf and Stiles (1966)
	Estuary Water		42(0 - 1.5); 23(14.5 - 17)	"
Cox-sackie	River Water with Some or Moderate Pollution	14(8 - 10); 5(20 - 23)		Clarke, Stevenson, and Kabler (1956)
	River Water with Gross Pollution	47(20 - 23)		"
	River Water (Autoclaved)	160(8 - 10); 102(20 - 23)		"
	Distilled Water	> 272(8 - 10); 100(20 - 23)		"
	Seawater in Laboratory		64(24)	Metcalf and Stiles (1966)
	Estuary Water		56(0 - 1.5); 32(14.5 - 17)	"
Echo	Seawater in Laboratory		> 130(5); 28(37)	"
	Estuary Water		50(0 - 1.5); 23(14.5 - 17)	"
Infec-tious Hepa-titis	Well Water (Contaminated)		Produced illness in volunteers after 10 weeks' storage	Neefe and Stokes (1945)

culture technique, and (3) ideally it should separate the virus from bacteria, protozoa, and other microbes, as well as toxic material to avoid the detrimental effect of the latter on tissue cells.

Various concentration methods have been reported in the literature. Older methods include precipitation, ion exchange, centrifugation and combination of these. Recent methods include hydro-extraction and two-phase separation (Shuvall et al., 1966, and Lund and Hedström, 1966), electrophoresis (Bier et al., 1966), continuous-flow ultracentrifugation (Anderson et al., 1966), passive hemagglutination (Smith and Courtney, 1966), concentration with polyethylene glycol (Cliver, 1966a), use of soluble ultrafilters (Gärtner, 1966), aluminum phosphate and aluminum hydroxide precipitation (Wallis and Melnick, 1966, 1967a), adsorption on membrane filters (Cliver, 1966b, and Wallis and Melnick, 1967b, 1967c), and adsorption on iron oxide (Rao et al., 1968). An older quantitative technique, grab sampling, has been made more practical by recent improvements in assay technology (Rawal and Godbole, 1964, and Berg et al., 1966), but the amount of water that can be sampled by this technique is limited because the water to be sampled for viruses is used to prepare the medium for nourishing the cell cultures that serve as the assay system.

The most commonly used method today is the gauze pad technique. Pads of gauze or sanitary napkins are suspended in flowing waters for several days. Pads are then squeezed after adjusting the pH of the absorbed water to eight, the expressed fluid centrifuged, first at low speed to sediment bacteria and particulate matter, and then at high speed to sediment viruses which are subsequently assayed. The major deficiency of this method is that it is not quantitative (Berg, 1968).

The method that is most promising at the moment for concentrating small quantities of viruses from large volumes of water is the membrane filter

technique of Cliver (Berg, 1968). The sample of water is filtered through a 0.45 μ Millipore membrane to which viruses adsorb. Presence of salts and a pH value close to neutrality facilitates virus adsorption (Berg, Dean and Dahling, 1968, and Wallis and Melnick, 1967b). Turbid waters can be cleared by filtration through course filters with relatively little loss of virus. Elution of the viruses is achieved by immersing the filters in three percent beef extract (Berg, Dean and Dahling, 1968). Viral density is then determined by conveniently inoculating cell cultures with the eluate. This technique, although the most quantitative available presently, suffers in that the beef extract is not a universal elutant.

The membrane filter technique is the most quantitative method available within its limitations. The gauze pad procedure is recommended for qualitative studies on the assumption that this technique may be the most sensitive available presently (Berg, 1968). This method has been recently used in the Santee Recreation Project, Santee, California (Merrell et al., 1967).

C. Removal of Viruses by Coagulation and Flocculation

The information on the removal of viruses in water by coagulation and flocculation is extremely sketchy and nonquantitative. This is mainly due to the lack of accurate quantitative assay techniques for animal viruses and a reliable method for concentrating water samples containing viruses. Many investigators have used bacterial viruses as models for studying this problem. When animal viruses were used, biological assays involving laboratory animals were employed instead of quantitative tissue culture technique. Furthermore, it is difficult to correlate the observations of different workers due to the fact that coagulation and flocculation conditions were different. In most of the studies undertaken in this area, attempts were made to arrive at quantitative values like percent virus removal, relationships between virus removal

and alum dosage, and settling time, etc. None of these studies was directed toward an understanding of the basic physico-chemical processes involved.

Using the virus of the mouse-adapted strain of human poliomyelitis, Carlson, Ridenour and McKhann (1942) found that flocculation using alum (100 ppm) did not render the water noninfective for mice, and that a rapid sand filter heavily impregnated with alum was somewhat more effective in removing the virus than the former process. Using both alum flocculation and filtration on water containing the virus of a monkey-adapted strain of human poliomyelitis, Kempf et al. (1942) freed the supernatant of the virus in two experiments and freed the effluent (sedimented and filtered) of the virus in one, out of the three experiments. Only by greatly increasing the alum dosage were they able to remove all the viruses. Since the data were measured by a biological assay involving the infecting of monkeys, there is some doubt that 100 percent removal was achieved even then. These authors also centrifuged their flocculated water and claimed that the supernatant could be freed of the virus if a floc sediment of 1.5 mg/l (in a Hopkins tube) was obtained with water flocculated at a dosage of eight grains/gal of alum; but the virus in the floc sediment was not destroyed. They could recover virus activity in the resuspended sediment when none was present in the supernatant. Working with the virus of Infectious Hepatitis, Neefe et al. (1947) found that alum flocculation and filtration through a diatomaceous earth filter did not completely remove the infective agent from the treated water. Forty percent of the human volunteers developed the disease after ingesting the treated water.

The basic mechanism involved in the removal of viruses by flocculation has been thought to be the formation of a metal cation (coagulant) - protein (virus) complex followed by precipitation and flocculation (Chang et al.,

1958b; Chang, Isaac and Baine, 1953; Felix, 1965; and Kabler et al., 1963). This hypothesis seems quite reasonable from the chemical aspects of coagulation. Stumm and Morgan (1962) observed:

> "The physical or double-layer theory has been developed in great detail and has, in its various forms of simplification, found wide acceptance....This theory has virtually replaced and superceeded the older chemical theory. These two coagulation theories are not, however, as mutually exclusive as they might appear to be on first sight, and it is important to call attention to the fact that purely chemical factors must be considered in addition to the theory of the double layer in order to explain, in a more quantitative way, the dependence of the stability of colloids upon the chemical composition of the medium....Occasionally, specific chemical equilibria, such as complex formation, may be more important than double layer compaction through counter-ion adsorption.... Complex formation reactions between aluminum or iron coagulant metal ions and carboxylic, phosphato, sulfato, or aromatic hydroxyl functional groups are important in the destabilization of such naturally occurring colloidal or dissolved impurities as color, proteins, and carbohydrate materials. The marked difference in the response of carboxyl, sulfate and phosphate colloids to coagulation by metal ions is indicative of specific chemical interactions."

By extrapolating the interactions of proteins and salts of metals to that of virus protein and aluminum, Chang, Isaac and Baine (1953) first postulated the aluminum-virus complex formation. Later, they showed the importance of this complex formation as the first stage in the removal mechanism by using Gum Arabic, a substance which interferes with flocculation (Chang et al., 1958b). Gummy substances form a protective coating on charged particles and are well known for their stabilizing effect on emulsions. Viruses are not destroyed as a result of the complex formation as was evidenced by the recovery of viruses from the floc fraction (Chang, Isaac and Baine, 1953, and Gilcreas and Kelly, 1955).

Chang, Isaac and Baine (1953) using bacterial virus showed that virus removal followed a Freundlich adsorption isotherm and that preformed floc had little effect on virus removal. In concentrating Coxsackie virus

suspensions by alum flocculation it was again noticed that virus recovery was not high when preformed floc was used (Stevenson et al., 1956). A linear relationship was obtained between percent removal/alum dosage and percent virus remaining in suspension for a defined pH, temperature, flocculation and sedimentation time. The percent removal was a function of coagulant dose below the upper limit of the "zone of flocculation." Robeck, Clarke and Dostal (1962) also noticed that increasing alum dosage increased the virus removal up to 99 percent. Polyelectrolytes were useful when filtration and coagulation were employed together. Furthermore, Chang, Isaac and Baine (1953) obtained different values of u in the relationship $R = uX^n$ (where R = percent removal/alum dose; u and n are constants and X = percent virus remaining in suspension) for two series of experiments conducted under identical experimental conditions. They concluded that some other unknown factor or factors were responsible apart from the cation-virus complex formation. As to the kinetics, they showed that 20 min were required for the first stage (complex formation). For the second step (aluminum-virus precipitate formation) they obtained a linear relationship between the log of virus remaining active and the square of the contact time. The energy of activation was calculated and found to be of the order of magnitude of that of diffusion (E = 6,770 calories).

In a recent publication, Berg (1964) reported 96 and 94 percent removal of Coxsackie A2 virus by alum and ferric chloride, respectively. Removal was 96.6 percent when both coagulants were used. Temperature in the range 5°C to 25°C did not affect the removal significantly. On the other hand, Chang noticed slightly lower removal in cold months (Senn et al., 1961). Virus removal ranging from 25 to 99 percent were reported by different workers (Senn et al., 1961). It is of interest that high virus removal by

Chang's group was achieved with low alum dosages, whereas low efficiency was attained by others who used high dosages. A critical comparison of virus removal efficiencies by flocculation cannot be made unless the conditions under which the experiments were conducted are known. As most of the investigators did not indicate the relative performance of the flocculation process as judged by visual inspection of turbidity removal, the relatively low virus removal reported might suggest that the flocculation process was inadequate.

Using a bacterial virus (bacteriophage against M. pyogenes var. albus) Chang, Isaac and Baine (1953) studied the fate of virus particles removed by flocculation. They found that virus particles were temporarily "inactivated" during the complex formation as a result of the formation of the aluminum salt of protein in the virus and would be "active" again when dissociated from the floc. They were able to "reactivate" the virus particles by redispersing the flocculated mixture at pH 7.6 with vigorous stirring. Sixty percent of the removed viruses could be recovered by this technique. Quite contrary to this observation, Puck, Garen and Cline (1951) demonstrated that trivalent cations like Al, Cr, and Fe permanently inactivate bacteriophages and their host cells. Later Yamamoto, Hiatt and Haller (1964) reported that $AlCl_3$ at concentrations between 10^{-5} and 2.10^{-3} M failed to inactivate bacteriophage MS2. Carlson et al. (1968) reported a significant increase in the percent inactivation of virus particles (bacteriophage T2) adsorbed on clay particles when aluminum salt was present in the system instead of calcium or sodium salts. However, no attempt was made to reactivate the virus particles or to assess the toxic effect of the aluminum on the virus or the effect of aluminum on the surface charge of the virus.

Other metal ions may have an important role in the removal of viruses by coagulation and flocculation, e.g. in slowing down the rate of aluminum-

virus complex formation. Interfering effects of calcium and magnesium were noticed by Chang et al. (1958b) during virus removal by two-stage flocculation with Ohio River water. They believed that the presence of calcium and magnesium ions in the raw water and the addition of CaO in the second stage interfered with the formation of the coagulant-cation bacterial-virus complex.

Little information is available regarding the influence of organic matter on the removal of viruses by flocculation. Using alum and artificially contaminated river water with Coxsackie virus Frankova, Cervenka and Symon (1964) obtained highest removal of infectivity when the virus was added in the form of a suspension of infected mouse brain and found that the optimum amount of alum for the removal of the virus was dependent on the concentration of organic matter in the water. However, Carlson et al. (1968) observed considerable reduction in percent virus (bacteriophage T2 and type 1 Poliovirus) inactivation by clays (Illite, Montmorillonite and Kaolinite in presence of sodium chloride or calcium chloride) when albumin or wastewater was present in the system. This was shown to be due to competition with virus for the adsorption sites on the clay.

In connection with their studies on the movement of viruses in ground water, Eliassen et al. (1966) observed the effects of synthetic polyelectrolytes on virus removal by alum flocculation. Virus removal was improved by the addition of varying concentrations of polyelectrolytes. Of the three species of polyelectrolytes tested, the results indicated that for similar dosages the cationic polyelectrolyte was approximately two orders of magnitude more effective than the anionic and neutral polyelectrolytes. Little virus removal was accomplished when the sand phase was absent in their jar tests. Presumably stirring and settling conditions were not sufficient for the floc to settle in the absence of the sorption effect of sand.

Quite contradictory observations have been reported regarding the effect of pH on Coxsackie and bacterial virus removal. Chang, Isaac and Baine (1953) found pH 5.5 to be the optimum and obtained very low removal at pH 7.0. In a later paper less removal was observed at pH 5.0 (Chang et al., 1958a). Quite different results were obtained with bicarbonate and phosphate buffers. Research conducted at Kings College in England (1956) reported 99 percent removal of bacteriophage with 60 ppm of ferric chloride at pH 6.5.

D. Removal of Viruses by Other Water Treatment Methods

(1) Filtration

A few observations have been made on filtration of viruses through sand and garden soil. Most of the earlier workers, as reviewed in the literature survey by Clarke and Chang (1959), obtained poor virus removal in laboratory studies in which the virus suspensions were usually prepared from infected animals. Presumably, the poor removal resulted from the high concentrations of extraneous organic matter in the suspensions.

The recent study of Robeck, Clarke and Dostal (1962) showed that one to 50 percent of the added Poliovirus was removed by rapid sand filters (2 to 6 gpm/sq ft) and 22 to 90 percent by slow sand filters (0.035 gpm/sq ft). However, rapid sand filtration of coagulated water, after adequate settling of the floc, removed 90 to 99 percent of the virus originally present. Using flocculated water, Gilcreas and Kelly (1955) obtained 35 and 98 percent removals for rapid sand (2 gpm/sq ft) and slow sand (0.2 gpm/sq ft) filters, respectively. The observations of Neefe et al. (1947) on the Infectious Hepatitis virus indicate insignificant removal of this virus by diatomaceous earth filtration.

(2) Disinfection

Viruses are more resistant than bacteria to chlorination. Different types

of enteric viruses vary widely in degree of resistance to free chlorine (Kabler et al., 1961, and Chang, 1968) and iodine (Berg, Chang and Harris, 1964).

The data of Weidenkopf (1958) on destruction of Poliovirus 1 by free chlorine at $0^{o}C$ revealed that HOCl was considerably more effective than the OCl^{-} ion. This is also supported by the data of Kelly and Sanderson (1958) on the destruction of enteroviruses. Poynter (1968) prepared a table from the data presented by Clarke et al. (1964) who, by collating data of their own with that of other workers, were able to produce what they considered should be reasonable estimates of the sensitivities of some of the enteroviruses and an Adenovirus relative to that of E. coli (Table 3).

E. Aqueous Chemistry of Aluminum

It is apparent from the solubility considerations of aluminum and iron that coagulation in water and wastewater treatment is carried out under conditions of pH and coagulant dosage such that the system is oversaturated with respect to the metal hydroxide (Stumm and O'Melia, 1968). However, it is thought that a brief discussion of the aqueous chemistry of aluminum is warranted here because a great majority of the experiments will involve soluble concentrations of aluminum.

Recently, studies on the hydrolysis of the aluminum ion in dilute aqueous solution have received increased attention. A substantial amount of new information concerning the specific chemical structures of the hydrolysis products of the aluminum ion have been obtained. According to the available literature on the aluminum hydrolysis reactions, solubility curves for aluminum have been calculated and presented (Black and Chen, 1967; Stumm, 1964; and Stumm and O'Melia, 1968). Figure 1 shows the aluminum solubility curve presented by Black and Chen (1967).

TABLE 3

EFFECT OF CHLORINE AND IODINE ON VIRUSES AND *E. coli*
(After Poynter, 1968)

Temperature °C	pH	Halogen		Time for 99 Percent inactivation (minutes)		Remarks
		Concentration mg/l	Form	*E.coli*	Virus	
25	7.0	9.0	Chloramine	-	30 (Poliovirus 1)	
25	7.0	0.5	Chloramine	-	420 (Poliovirus 1)	
5	-	0.1	Mono-chloramine	490		
5	10.7	0.1	OCl^-	>110		Trace HOCl probably present
0-6	-	0.1	HOCl		0.55 (Adenovirus 3)	
0-6	-	0.1	HOCl	1.66	8.50 (Poliovirus 1)	
0-6	-	0.1	HOCl		40.00 (Coxsackie A2)	
5	-	1.0	I_2	1.0	1100.00 (Coxsackie A9)	Most resistant virus tested
5	-	1.0	I_2		110.00 (Echo 7)	Most sensitive virus tested

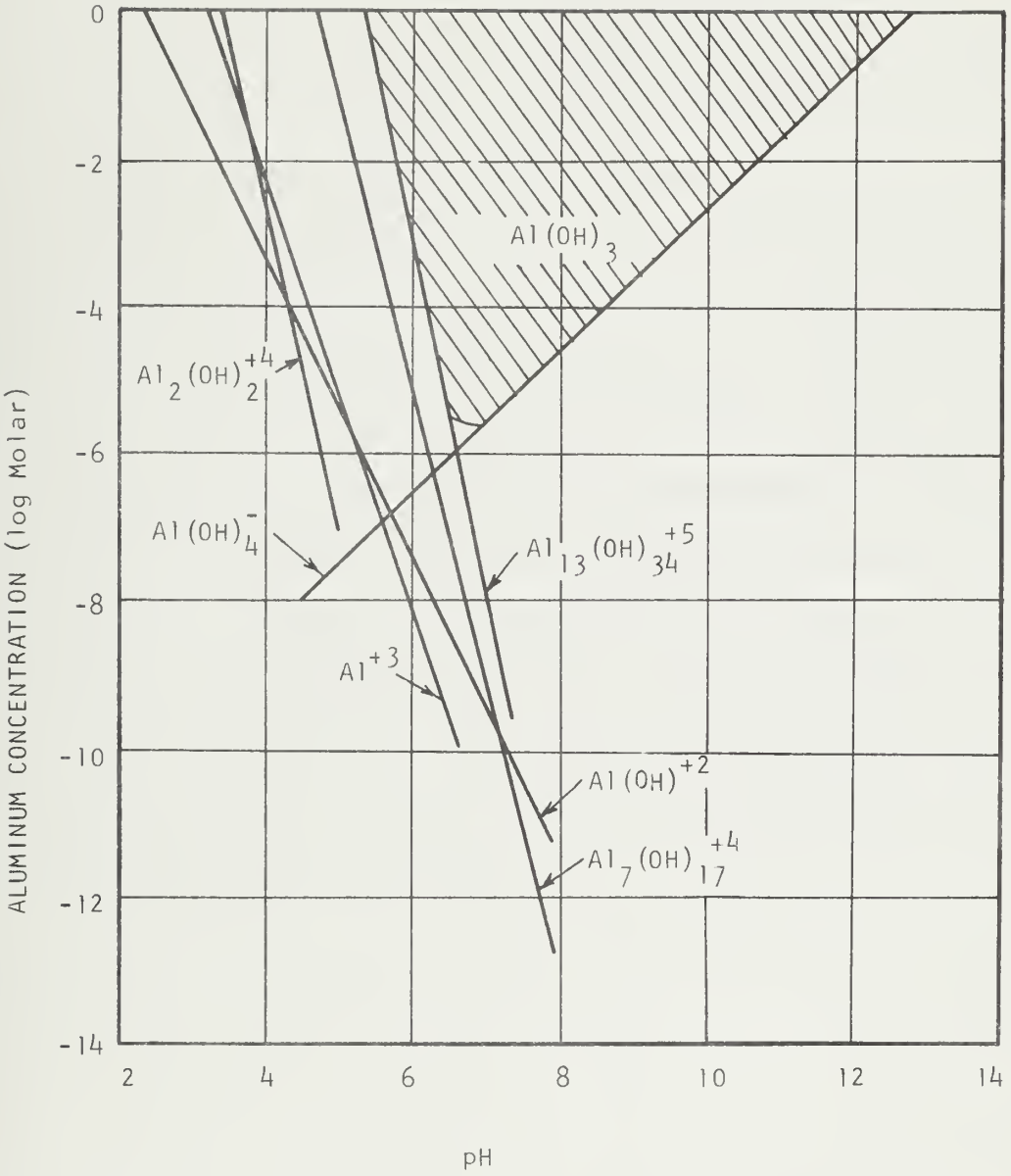

FIGURE 1. SOLUBILITY CURVE FOR ALUMINUM HYDROXIDE.
(After Black and Chen, 1967).

Figure 1 is useful for visualizing the specific regions of various aluminum ion species for varying pH values at different total concentrations of aluminum. Within a range of total aluminum ion concentration from 10^{-6} M to 10^{-4} M, the pH scale from 3 to 10 can be roughly divided into four different regions in which different predominant aluminum ion species are present. In the region below pH 4, the hydrated trivalent aluminum ion is the most active ion species. In the region between pH 4 and 6, the predominant aluminum ion species are the hydrolyzed polymeric multivalent cation species: $Al_7(OH)_{17}^{+4}$ and $Al_{13}(OH)_{34}^{+5}$. Predominance of other cation species, such as $Al_6(OH)_{15}^{+3}$ and $Al_8(OH)_{20}^{+4}$ have also been proposed in the literature (Black and Chen, 1967). In the pH range roughly from 6 to 8, insoluble aluminum hydroxide, $Al(OH)_3$, is the predominant species, and the aluminate ion, $Al(OH)_4^-$, is believed to predominate above pH 8. However, it is interesting to note that, in the region between pH 5 and 8.5, the formation of insoluble aluminum hydroxide colloids or precipitates does not take place up to a total aluminum concentration of about 10^{-4} M as evidenced by the Tyndall effect in aluminum sulfate solution (Black and Chen, 1967, and Morgan, 1967).

III. SCOPE OF THE INVESTIGATION

From the preceding discussion, it is evident that there is a great need to obtain more information on the basic mechanisms involved in virus removal by chemical coagulation and flocculation so as to enable the engineer to put the process on a sound scientific base. The removal of viruses from water supplies is extremely important and, becomes more so, with the potential need for wastewater reuse. Information on the basic mechanisms involved in virus removal by coagulation and flocculation should contribute significantly to the solution of the problem and aid in the development of sound design standard for their removal in water treatment facilities.

Most of the investigations so far undertaken in this area have been directed towards quantitative results, i.e. gross removal efficiencies. Very limited attention has been given to the mechanisms of virus removal (Chang et al., 1958b; Chang, Isaac and Baine, 1953; Felix, 1965; and Kabler et al., 1963). Considering the observations of the past researchers in this area and the interaction of proteins with metals ions, the virus removal mechanism may be postulated as a two-stage reaction. The first stage is the formation of a virus-aluminum complex, the second stage is the subsequent precipitation and flocculation of the complex. Basically, the first stage is the interaction of virus with metal ion. The present research was initiated to investigate this interaction. The study was undertaken along the following lines:

a. demonstration of a "complex formation" between viruses and aluminum, and

b. nature of the "complex" and virus inactivation by alumimum.

The second phase of the study was directed towards a quantitative study of virus removal by alum flocculation under controlled laboratory conditions.

The effects of the following variables in virus removal by chemical coagulation and flocculation were also investigated:

a. pH and coagulant dose,

b. bivalent metal ions like calcium and magnesium,

c. organic matter, and

d. synthetic polyelectrolytes (coagulant aids).

IV. MATERIALS AND METHODS

A. Materials

(1) Viruses and Preparation

One of the main criteria in selecting a virus for this study was feasibility of culturing and enumeration. Assay techniques for bacterial viruses are better developed than for animal viruses. Bacterial virus assays require about 12 to 24 hr compared with 5 to 10 days for animal viruses, and culture procedures are simpler for bacterial hosts. Bacterial and animal viruses have many similar physical, chemical and biological properties, i.e. size, net electric charge, protein coating, etc. (Adams, 1959). Thus, it may be assumed that their differences in behavior when subjected to coagulation and flocculation may not be much greater than the differences in these properties among the animal viruses alone, which could cause significant variations in removal by chemical coagulation and flocculation. Furthermore, there is much more known about the composition and properties of bacterial viruses than is known about the animal viruses. This allows for a more detailed examination of their behavior in chemical coagulation.

Two bacterial viruses, bacteriophages T4 and MS2 against Escherichia coli, were selected as the model viruses for this study. Bacteriophage T4 is a DNA (deoxyribonucleic acid) containing virus whereas bacteriophage MS2 is a RNA (ribonucleic acid) containing virus. Bacteriophage T4 was selected as the principal model virus because of its stability in agitated systems and its greater ease of culturing and enumeration (Cookson, 1966, and Drake, 1967). Bacteriophage MS2 was selected as the second model virus in order to confirm the results with bacteriophage T4. It was thought that use of MS2 along with

T4 in some of the major experiments would allow the data to be interpreted in terms of viruses which may be more significant in water supplies. MS2 was selected because of its resemblance in size and shape, and the type of nucleic acid to poliovirus (Kruse, 1968; Hanson, 1969; and Spiegelman, 1969).

a. Escherichia coli Bacteriophage T4

Bacteriophage T4 (Figure 2) and its host E. coli BB were obtained from Dr. John W. Drake, Department of Microbiology, University of Illinois. Bacteriophage T4 has the following properties (Table 4) (Kellenberger, 1962; Stent, 1963; and Putnam, 1953).

TABLE 4

PROPERTIES OF E. coli BACTERIOPHAGE T4

Size	
head	65 x 95 mμ
tail	20 x 95 mμ
Specific weight	3.3×10^{-16} gm/particle
pH stability range	5.0 - 9.0
Sedimentation constant	700 - 1000 Svedberg units ($S_{20,w}$)
Nucleic acid	DNA (deoxyribonucleic acid)

The procedure for growing bacteriophage T4 was also obtained from Dr. John W. Drake. Stock suspensions were prepared by infecting an early log phase broth culture of E. coli BB with bacteriophage T4 at a low multiplicity of infection (approximately 0.02 phages/ml). The infected culture was incubated at 37°C until the lysis was complete as evidenced by visible reduction of turbidity. Few drops of chloroform were then added to the lysed culture and

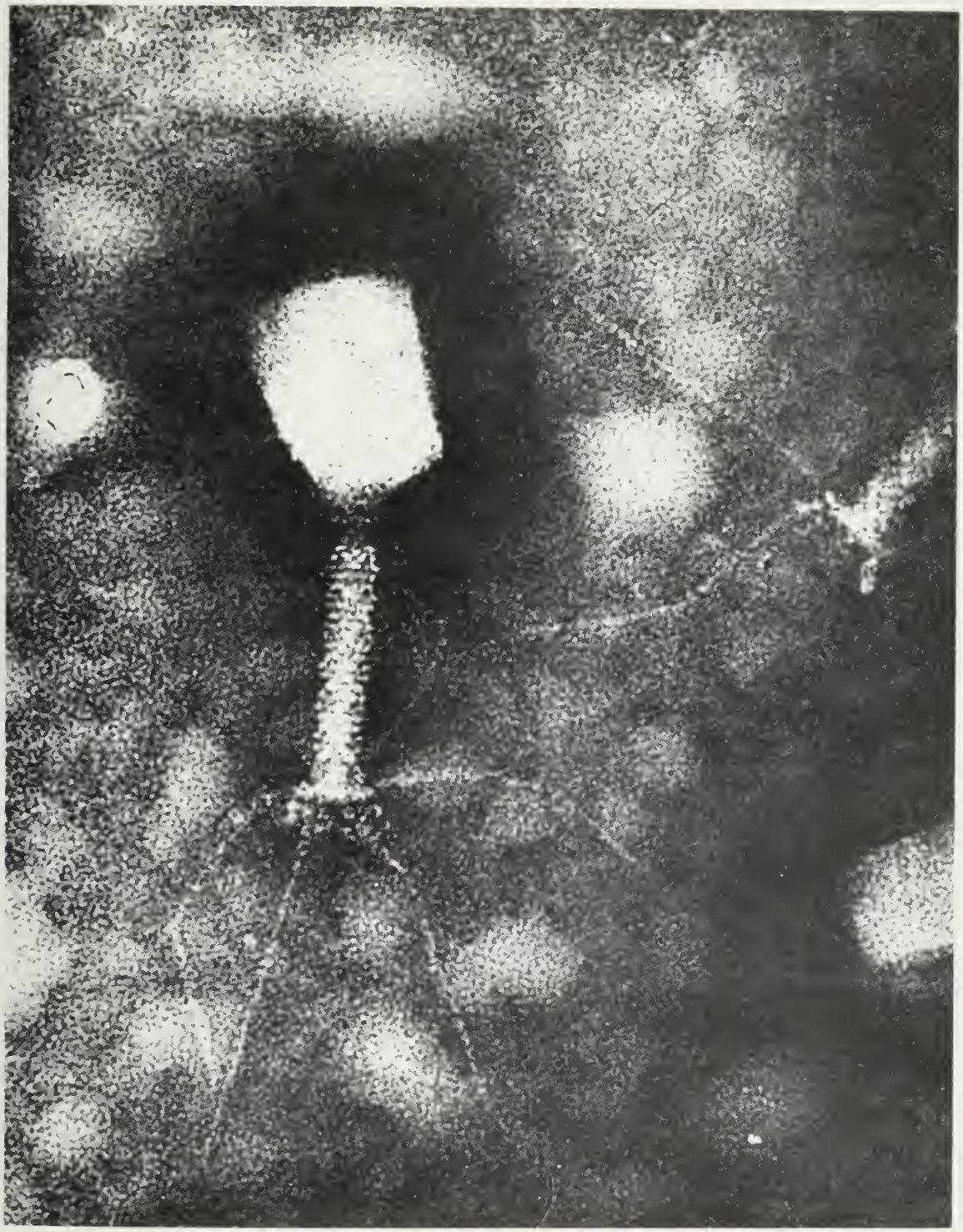

FIGURE 2. ELECTRON MICROGRAPH OF BACTERIOPHAGE T4.

Final magnification 420,000 X. Provided by T. F. Anderson, The Institute for Cancer Research, Philadelphia, Pennsylvania.

mixed well in a vortex mixer in order to facilitate lysis of the unlysed cells. The suspension was centrifuged at low speed (5,900 x g for 10 min at 4°C) to remove bacterial cells and cell debris followed by high speed centrifugation (34,800 x g for one hr at 4°C) to sediment the virus particles. The sedimented pellet was finally resuspended in phage buffer. The final purification step was repeated twice. The purified stock was then stored at 4°C. Stocks prepared in this way usually titered between 10^{10} - 10^{11} phages/ml.

b. Escherichia coli Bacteriophage MS2

A purified stock suspension of bacteriophage MS2 (Figure 3) and its host E. coli A19 were originally obtained from Dr. S. Spiegelman, Department of Microbiology, University of Illinois. Later, purified stock suspension of MS2 in 0.05 M tris buffer (pH 7.6) were purchased from Miles Laboratories, Inc., Elkhart, Indiana. Bacteriophage MS2 has the following properties (Table 5) (Overby et al., 1966).

TABLE 5

PROPERTIES OF E. coli BACTERIOPHAGE MS2

Particle diameter	25 mμ
Molecular weight	3.7×10^6 gm
Isoelectric point (pH)	3.9
Sedimentation constant	79 Svedberg units ($S_{20,w}$)
Nucleic acid	RNA (ribonucleic acid)

(2) Biological Media

a. Media for Escherichia coli Bacteriophage T4

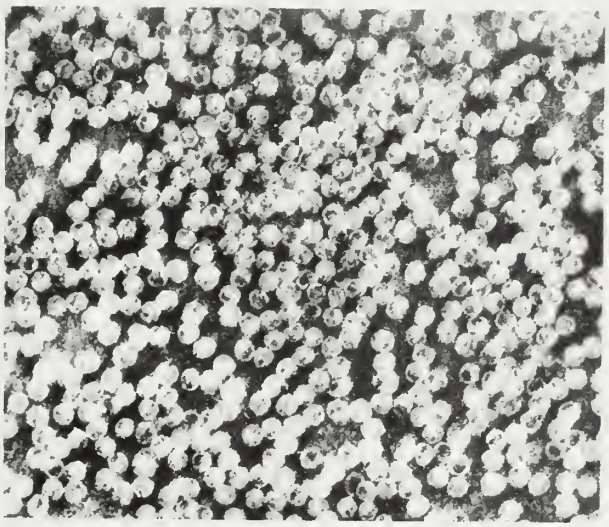

FIGURE 3. ELECTRON MICROGRAPH OF BACTERIOPHAGE MS2.

Final magnification 100,000 X. Provided by S. Spiegelman, University of Illinois, Urbana, Illinois.

T Agar (T4):

(Constituents per liter of water)

Bacto-Tryptone (Difco)	10.0 gm
Bacto-Agar (Difco)	12.0 gm
NaCl	5.0 gm

L Broth (T4):

(Constituents per liter of water)

Bacto-Tryptone (Difco)	10.0 gm
Yeast Extract (Difco)	5.0 gm
NaCl	10.0 gm
Glucose	1.0 gm

Adjusted to pH 7.0 with 1 N NaOH

Soft Agar (T4):

200 ml T agar (T4) plus 175 ml L broth (T4)

Phage Buffer (T4):

(Constituents per liter of water)

Tris(hydroxymethyl)aminomethane	1.21 gm
$MgSO_4$	0.60 gm
NaCl	5.85 gm

Adjusted to pH 7.4 with 1 N HCl

b. Media for Escherichia coli Bacteriophage MS2

L Broth (MS2):

Same as L broth (T4) plus 1.0 ml 2 M $CaCl_2$ per liter

L Agar (MS2):

L broth (MS2) plus 15 g/l Bacto Agar (Difco)

Soft Agar (MS2):

L broth (MS2) plus 10 g/l Bacto Agar (Difco)

Phage Buffer (MS2):

(Constituents per liter of water)

Tris(hydroxymethyl)aminomethane	6.06 gm
NaCl	5.85 gm

Adjusted to pH 7.6 with 1 N HCl

(3) Coagulant and Polyelectrolytes (Coagulant Aids)

The coagulant used in this study was aluminum sulfate $[Al_2(SO_4)_3.18\ H_2O]$ marketed by Allied Chem. Corp., Morristown, N. J.

The polyelectrolytes (coagulant aids) used are listed in Table 6. Two cationic and two anionic polyelectrolytes were selected. Stock and working solutions were prepared according to the instructions given in the product literature.

TABLE 6

POLYELECTROLYTES (COAGULANT AIDS)

Name	Type	Manufacturer
Primafloc C-7	Cationic (polyamine)	Rohm and Haas
Catfloc	Cationic	Calgon Corp.
Primafloc A-10	Anionic (polycarboxylic)	Rohm and Haas
Coagulant Aid #243	Anionic	Calgon Corp.

(4) Water

Distilled water demineralized through two Illco-Way Research Model Demineralizer (Illinois Water Treatment Co., Rockford, Illinois), and then

sterilized by autoclaving at 15 psi for 15 min was used in all soluble aluminum studies. Sterilized laboratory deionized water was used in preparing the raw water for the coagulation and flocculation studies (jar tests).

(5) Organic Materials

The following organic materials were used in this study:

a. Bovine Serum Albumin (5 percent). Prepared by Pentex Inc., Kankakee, Illinois, and distributed by Calibiochem, Los Angeles, California.

b. Egg Albumin. Prepared by Allied Chem. Corp., Morristown, N. J.

c. Beef Extract. Prepared by Difco Laboratories, Detroit, Michigan.

d. Wastewater and wastewater effluents. Collected fresh from the Urbana-Champaign Sanitary Distrist waste treatment plant and prepared by filtering through four layers of cheesecloth so as to remove the larger size particles.

(6) Glassware

All glassware used for the soluble aluminum studies were cleaned by soaking overnight in chromic acid followed by rinsing in tap water and deionized water. Glassware used for the coagulation and flocculation studies (jar tests) were cleaned by soaking overnight in Haemo-sol (Meinecke and Co., New York, N. Y.) followed by rinsing in tap water and deionized water, as suggested by Chang, Isaac and Baine (1953). Sterilization was accomplished in a hot air oven at 200°C for one hr or longer.

B. Chemical and Biological Assay Techniques

(1) Determination of Aluminum

A simple, rapid and sensitive method for determining soluble aluminum concentrations in the microgram range was required. The eriochrome cyanine R method originally introduced by Knight (1960) and later modified by Shull and Guthan (1967), and Chaudhuri and Engelbrecht (1968) was used. The standard

aluminum curve shown in Figure 4 was used to determine the aluminum concentration in unknown samples.

In the procedure a 15 ml sample was used. The sample was first titrated to pH 4.5 with N/50 sulfuric acid and then one ml added in excess. This was followed by the addition of one ml of ascorbic acid (1 g/l) and 10 ml of buffer solution (136 gm sodium acetate plus 40 ml of 1.0 M acetic acid in one liter of deionized water). Finally 5 ml of 0.03 percent eriochrome cyanine R was added, mixed and immediately made up to a volume of 50 ml with deionized water. The sample was mixed again and allowed to stand for 5 to 10 min. The absorbance was read against a suitable blank on a spectrophotometer (Universal Spectrophotometer, Model 14, Coleman Instruments, Inc., Maywood, Illinois) using a wave length of 535 mμ and a 4 cm light path. All readings were corrected for dilution due to added solutions.

(2) Assay Procedure for Bacteriophages

The assay procedure for bacteriophage T4 was obtained from Dr. John W. Drake. Before assaying, the sample was diluted in L broth (T4) to yield about 200 plaques per plate. A liquid top-agar mixture was prepared from 2.5 ml of soft agar (T4) at 45°C, 0.25 ml of a log growth phase culture of E. coli BB cells, and 0.1 ml of the diluted virus sample. The top-agar mixture was then poured on solidified bottom agar (T agar) plates and incubated at 37°C for 12 to 24 hr. "Plaques" were counted with the aid of a Quebec colony counter. Triplicate plates were prepared from each sample to increase accuracy.

The assay procedure used for bacteriophage MS2 was very similar to that of bacteriophage T4. E. coli A19 was the indicator bacteria. The top agar was soft agar (MS2) and the bottom agar was L agar (MS2). Plates were incubated at 37°C for 6 to 8 hr before counting.

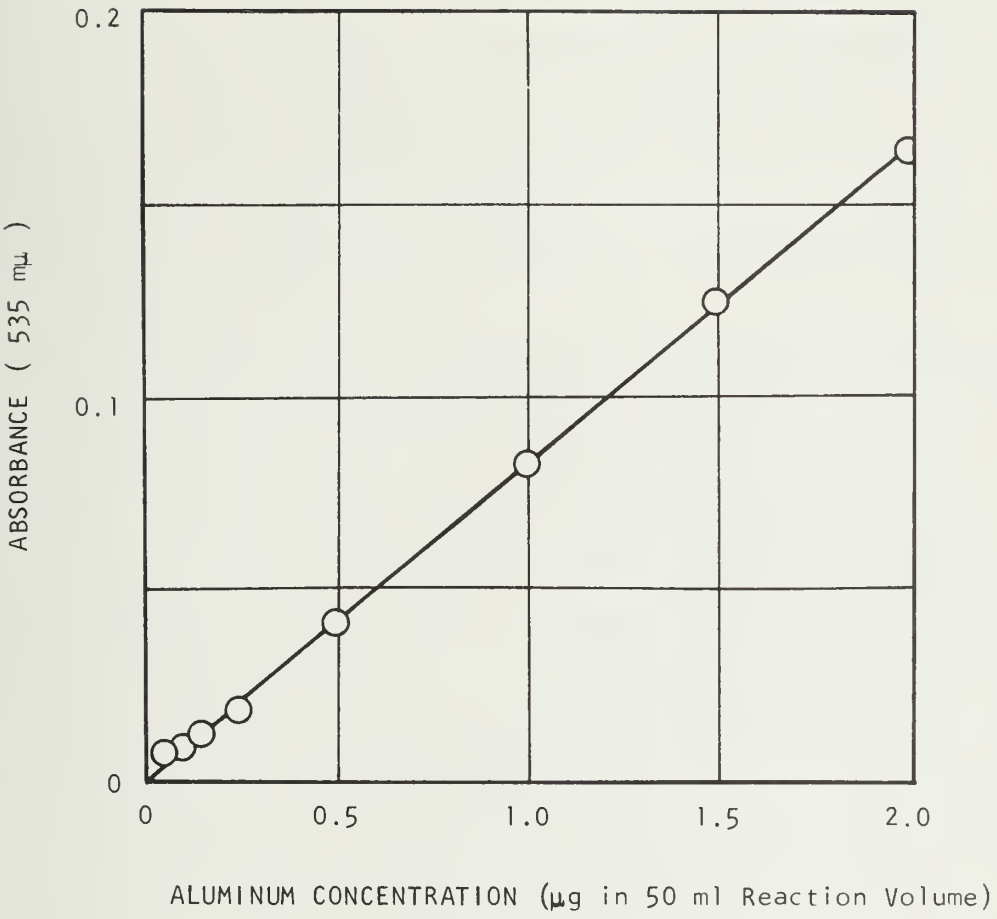

FIGURE 4. STANDARD CURVE FOR ALUMINUM.

C. Experimental Technique and Equipment

(1) Kinetics of Adsorption of Aluminum by Viruses

All studies on the kinetics of adsorption of aluminum involved soluble concentrations of aluminum. Concentrations of aluminum were always kept below the solubility at the pH of the test (Figure 1). Experiments were carried out at three different pH values: pH 5.0 (0.2M acetate buffer), pH 6.0 (0.2M succinate-sodium hydroxide buffer), and pH 9.0 (0.2M borate-potassium chloride-sodium hydroxide buffer). All experiments were performed at room temperature (24°C to 25°C).

In the procedure the reaction mixture (total volume 150 ml) was prepared by adding 95 ml of deionized water, 20 ml of buffer solution, 25 ml of aluminum solution (1 ml = 0.25 µg aluminum) to give a final concentration of 1.54×10^{-6}M, and 10 ml of purified virus stock suspension to yield a final virus concentration of $3\text{-}6 \times 10^{10}$/ml. A control was prepared to which 10 ml of phage buffer was added instead of the virus stock suspension, and a reagent blank consisting of 120 ml of deionized water, 20 ml of buffer solution, and 10 ml of phage buffer. Immediately after adding the virus stock suspension, the reaction mixture was mixed well and 20 ml samples were withdrawn periodically and immediately filtered through a 0.45µ membrane filter (Millipore Corp., Bedfore, Mass.). The first sample was withdrawn immediately after mixing. Subsequent samples were withdrawn at 2, 5, 10 and 15 min. In the filtration procedure, the first 5 ml of the filtrate were wasted; the remaining 15 ml were saved for the determination of aluminum. Aluminum concentrations in these filtrate samples were determined by the eriochrome cyanine R method using a filtered reagent blank. Aluminum concentrations were also determined in duplicate 15 ml filtered samples from the control. The difference in aluminum concentrations between this and that in a filtered sample of the

reaction mixture was used to compute the quantity of aluminum adsorbed by the virus particles. The average of duplicate aluminum determinations on the unfiltered 15 ml samples from the control flask was denoted as "available aluminum." This available aluminum concentration was always found to be slightly lower than the calculated concentration added. This indicated that some loss of aluminum from the liquid phase was taking place. It is believed that this was due to the adsorption on glassware. A typical experimental protocol with accompanying calculations is shown in Table 7.

TABLE 7

EXPERIMENTAL PROTOCOL FOR KINETIC STUDIES

	Deionized Water (ml)	pH Buffer (ml)	Aluminum Solution (ml)	Virus Suspension (ml)	Phage Buffer (ml)
Reagent Blank	120	20	-	-	10
Reaction Mixture	95	20	25	10	-
Control	95	20	25	-	10

Aluminum adsorbed by the virus particles = (Aluminum concentration in filtered sample from the control) - (Aluminum concentration in filtered sample of the reaction mixture)

Available aluminum = Aluminum concentration in unfiltered sample from the control

Filtration through a 0.45μ Millipore membrane filter was used as a means for rapid physical separation of the virus particles from the reaction mixture. Viruses are known to adsorb on 0.45μ Millipore membrane filters (Cliver, 1966b, and Wallis and Melnick, 1967b, 1967c). Further, it has been demonstrated that E. coli bacteriophages adsorb on 0.45μ Millipore membranes (Hoff and

Jakubowski, 1965, and Loehr and Schwegler, 1965). Preliminary tests indicated that under the experimental conditions used in this study, not more than 9 to 10 percent of the virus particles were passing through the membrane filters.

(2) Virus Inactivation Studies

Experiments to evaluate inactivation of bacteriophages T4 and MS2 in the presence of soluble concentrations of aluminum were carried out at pH 5.0 (0.2 M acetate buffer) and at room temperature (24°C to 25°C). Experimental tubes were prepared by adding 3.3 ml of buffer solution, 1.0, 5.0 or 7.0 ml of aluminum solution (1 ml = 0.25 µg aluminum), 0.5 ml of a suitable dilution of the virus stock suspension to yield a final virus concentration of 10^6 - 10^7/ml, and deionized water to make up to a final volume of 25 ml. A control tube was also prepared without any aluminum. Samples for virus assay (0.1 ml) were withdrawn at 0, 3, 6, 12, and 24 hr and the virus titer determined by the standard technique. Duplicate experimental tubes were also prepared in which 0.5 ml of phage buffer was added instead of the virus dilution. Samples were withdrawn from each of these tubes for determination of "available aluminum."

(3) Quantitative Studies on Adsorption of Aluminum by Viruses

a. Studies on Adsorption of Aluminum by Viruses

These studies were carried out at pH 5.0 (0.2 M acetate buffer) and at room temperature (24°C to 25°C). A procedure similar to that used in the kinetic studies was followed for preparation of reaction mixtures, blanks, and control flasks. Virus concentrations were varied from 2 x 10^{10}/ml to 1.8 x 10^{11}/ml. Since it was evident from the kinetic studies that adsorption of aluminum by the virus particles was very rapid and took place at least within the first 30 to 40 sec, samples from the reaction flasks were withdrawn and filtered 5 to 10 min after the start of the reaction. The quantity of aluminum

adsorbed by virus particles and "available aluminum" were computed in the same manner as before.

b. Aluminum Saturation Curves

Experiments were also carried out to obtain saturation curves for aluminum adsorption by different concentrations of bacteriophage T4. Studies were made at pH values of 5.0 (0.2 M acetate buffer), 6.0 (0.2 M succinate-sodium hydroxide buffer), and 9.0 (0.2 M borate-potassium chloride-sodium hydroxide buffer), and at room temperature (24°C to 25°C). Using a procedure similar to the one used in the studies on adsorption of aluminum by viruses, quantities of aluminum adsorbed by a particular concentration of bacteriophage T4 particles at varying "available aluminum" concentrations were computed and plotted.

(4) Quantitative Studies on Virus Removal by Chemical Coagulation and Flocculation (Jar Tests)

A bench-scale apparatus employing a carbonic acid-bicarbonate buffer system for pH control was set up in the laboratory for coagulation and flocculation studies (Figure 5). Bicarbonate buffer was used because this is the main buffering system present in natural surface waters (Chang et al., 1958a). All experiments were performed at room temperature (24°C to 25°C).

Mixing was provided with a six-place multiple stirrer (Phipps and Byrd, Richmond, Va.). The tachometer readings were found to agree with paddle rpm values. Specially constructed paddles (Figure 6), made from 0.25 in. stainless steel rod, were used in an attempt to provide more uniform velocity gradients than is thought to occur with the conventional 1 x 3 in. paddles. The paddles were cleaned and sterilized before each experiment using the standard procedure for cleaning glassware.

Control of pH, over the range of 8.3 to 5.0, was obtained by introducing various mixtures of air and CO_2 into the partially confined atmosphere above

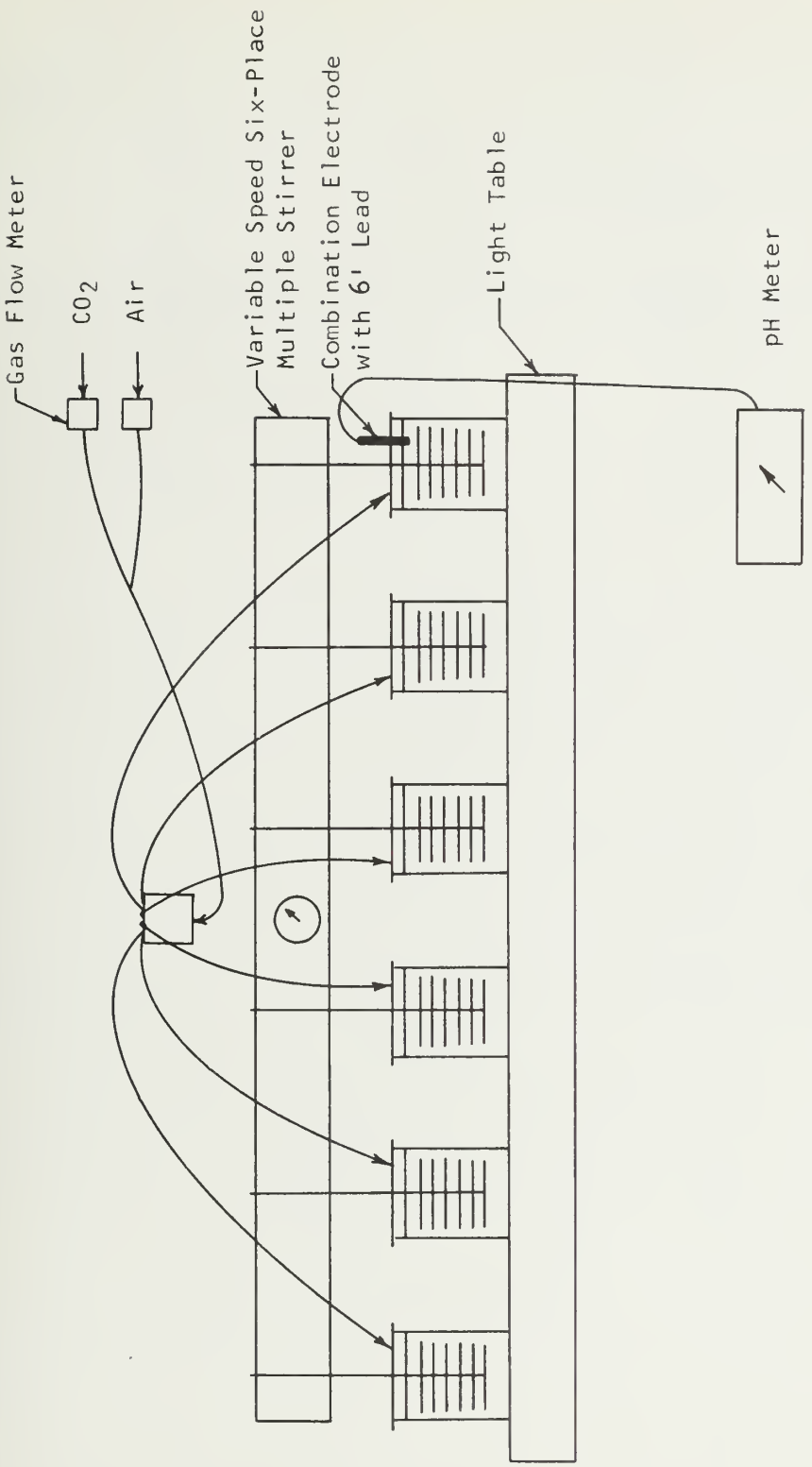

FIGURE 5. SCHEMATIC DRAWING OF APPARATUS FOR COAGULATION AND FLOCCULATION STUDIES (JAR TESTS).

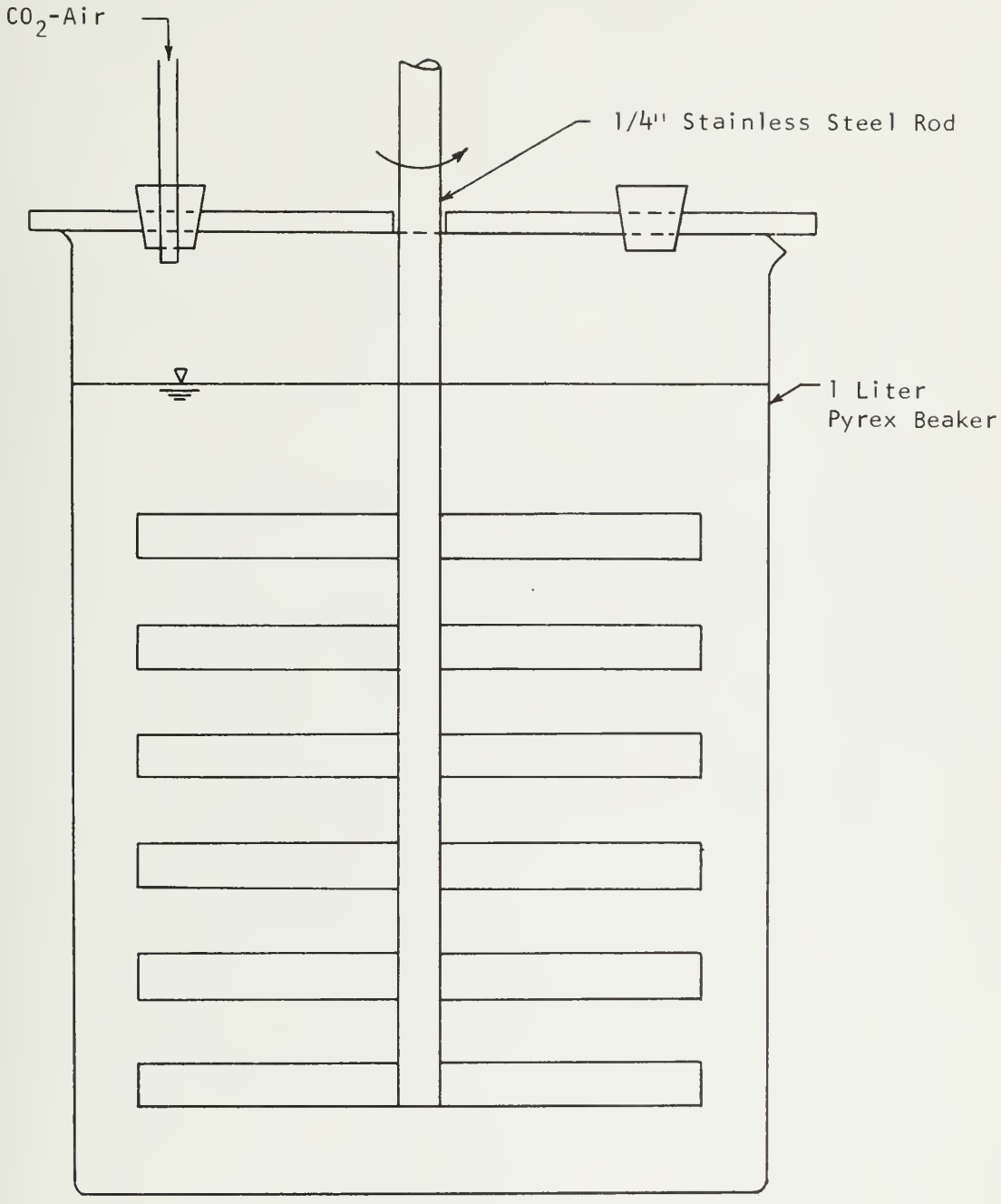

FIGURE 6. SCALE DRAWING OF REACTION VESSEL FOR COAGULATION AND FLOCCULATION STUDIES (JAR TESTS).

each beaker. By measuring the individual gas-flow rates prior to blending, various mixtures of the two gases could be obtained. The blended gas was distributed from a central manifold to the individual beakers. Each experiment was initiated when an equilibrium pH was achieved; this usually required 0.5 to one hr.

Values of pH were determined in individual beakers before the addition of coagulant and after the settling period. A Beckman Electromate pH meter was used. It was standardized daily using commercial buffer solutions of pH 4, 7, and 9.

The "raw" water was made up in 10 liters batches in a covered polyethylene vat. The raw water for all experiments contained 150 mg/l of sodium bicarbonate, 120 mg/l of Montmorillonite (Wyoming Bentonite), and 4-5 x 10^5/ml of bacteriophage T4 or MS2. The clay was supplied by Ward's Natural Science Establishments, Inc., Rochester, N. Y. Montmorillonite was selected because it has been shown to be present in natural waters (Packham, 1962).

Samples for turbidity measurement, 30 ml each, were taken with a 4 mm bore pipet, 1.5 in. below the air-water interface and delivered to 50 ml beakers. All turbidity measurements were made after the samples had been in the instrument (Model 1860, Hach Chemical Co., Ames, Iowa) for two min.

Samples for virus enumeration, 0.1 ml each, were taken with 0.1 ml serological pipets, 1.5 in. below the air-water interface and immediately diluted in L broth for subsequent assay by the standard assay procedure for bacteriophages.

The following method was used in performing an experiment: (1) six 975 ml aliquots of the "raw" water were placed in individual beakers situated on the mixing apparatus, (2) the six beakers were mixed simultaneously as pH was adjusted, (3) a calculated quantity of water was added to each beaker to

give a final total volume of one liter after the addition of chemicals, (4) while mixing rapidly at 100 rpm, a stoichiometric amount of sodium bicarbonate was added to each experimental beaker to neutralize the acidity due to aluminum sulfate added in the next step and then chemicals (aluminum sulfate, polyelectrolytes, etc.) were added into all but one beaker which served as a blank, and (5) mixing was continued at 100 rpm for one min followed by 30 min of slow mixing at 20 rpm. Mixing was stopped gradually over a 40 sec period and the mixing paddles were left in place during the settling period. Samples for virus assay and turbidity measurements were withdrawn after 30 min of quiescent settling. The pH of each system was determined, also. In experiments using coagulant aids, rapid mixing was extended for a period of one min after the polyelectrolyte addition, as recommended by the manufacturer.

(5) Virus Recovery from Settled Floc

After performing a jar test at the optimum pH and aluminum sulfate dosage for the particular virus, contents of both the blank and the experimental beakers were stirred at a high speed for 15 min using a magnetic stirrer so as to disperse the floc completely. While being stirred, duplicate 5 ml aliquots were withdrawn from each beaker with a broken-tip pipet and poured into screw-cap tubes containing 15 ml of an elutant. Deionized water, 3 percent beef extract, one percent bovine serum albumine, 0.1 M tris buffer (pH 8.0), and 0.2 M phosphate buffer (pH 8.0) were used as elutants. Tubes were kept at 4°C with the contents mixed every 15 min in a vortex mixer. After 6 hr the content of each tube was centrifuged at 5,900 x g for 10 min and the supernatant assayed for the virus titer. Recoveries were calculated on the basis of the virus titer of the supernatant from the blank tube.

V. RESULTS AND DISCUSSION

Results of all experiments are presented in graphical or tabular form. In order to facilitate the presentation, a discussion of the results follows each phase of the experimental work. For the most part, only typical results are shown and discussed although all results were substantiated by two or more replicate experiments.

The terms "coagulation" and "flocculation" often denote different meanings. Recently there has been shown an awareness of the long existing general confusion in the literature over the meaning of these two terms. It should be recognized that in the overall process of coagulation and flocculation there is a cause-and-effect relationship. For reactions of the type which are encountered in water treatment practice, destabilization and particle collision opportunity can be viewed as "causes." Aggregation of the destabilized, colliding particle is then an "effect." In order for a conventional water treatment plant to operate effectively both destabilization (accomplished by chemical addition) and particle collisions (accomplished primarily by mixing) must be provided. In presenting the results of this study, "coagulation" will refer to destabilization and "flocculation" will refer to aggregation. When used together, these two terms will denote destabilization and aggregation as provided in a water treatment plant and will be referred as "the process of coagulation and flocculation."

A. Adsorption of Aluminum by Viruses

(1) Kinetics of Adsorption

Kinetics of adsorption of aluminum at pH values 5, 6, and 9 by bacteriophages T4 and MS2 are shown in Figures 7 and 8, respectively. Attempts were

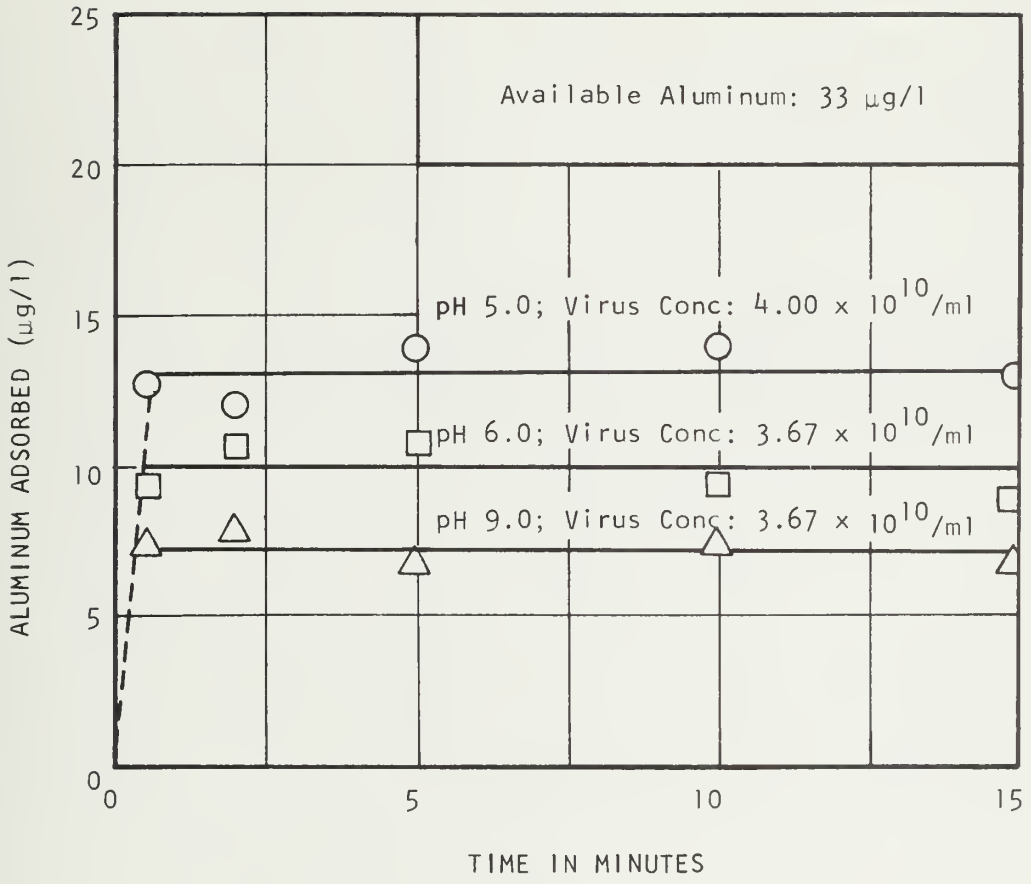

FIGURE 7. KINETICS OF ADSORPTION OF ALUMINUM BY BACTERIOPHAGE T4.

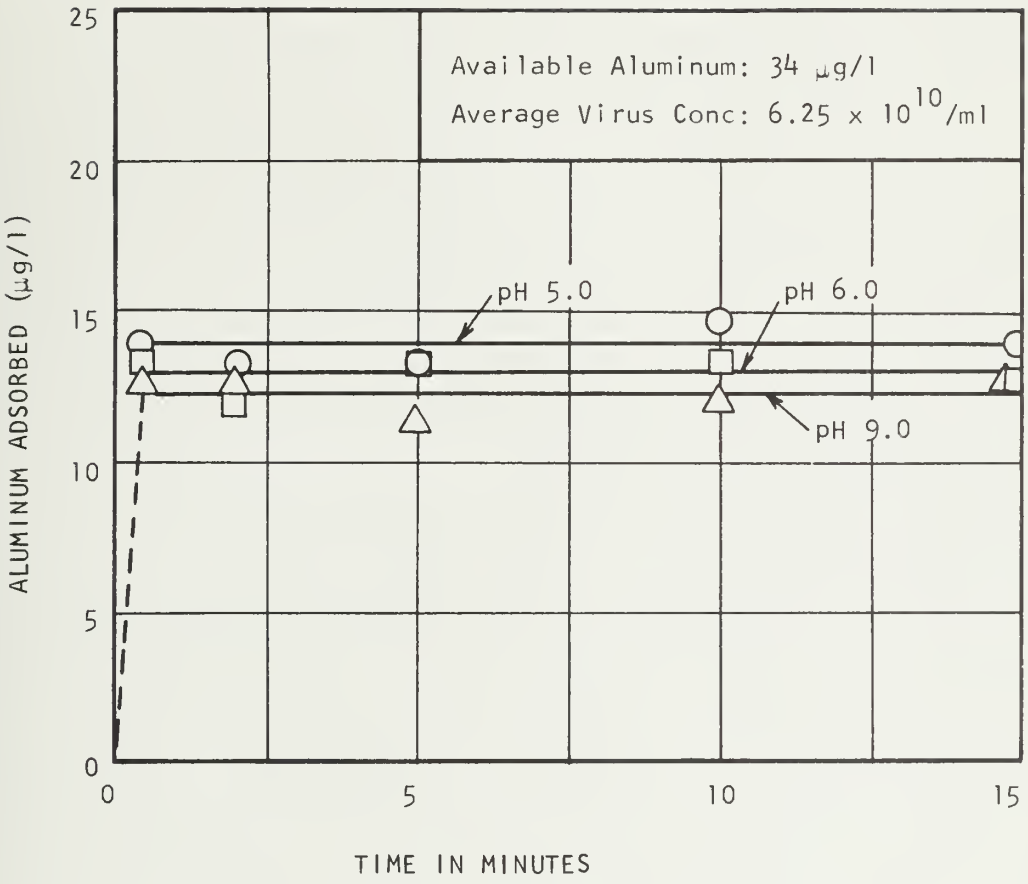

FIGURE 8. KINETICS OF ADSORPTION OF ALUMINUM BY BACTERIOPHAGE MS2 .

made to keep the virus concentrations constant for a particular bacteriophage in each system. However, it is to be borne in mind that the virus concentrations reported here are as determined by the plaque count method and do not indicate the total number of actual virus particles in the sample. The latter can be determined only by an electron micrographic count (Luria, Williams and Backus, 1951). Although specific information is lacking, it is generally accepted that the precision of the plaque count method is on the order of 5 to 10 percent (Drake, 1967). Since processing of the sample took approximately 30 to 40 sec before the virus particles were physically separated from the reaction mixture, the data for the samples withdrawn from the reaction mixture immediately after mixing were plotted against 30 sec.

It is evident from Figures 7 and 8 that adsorption of aluminum by virus particles is very rapid and takes place at least within the first 30 to 40 sec. Maximum adsorption is also attained at this time. For all practical purposes it may be regarded that adsorption of aluminum by virus particles is instantaneous. However, Chang, Isaac, and Baine (1953) estimated that 20 min would be required for aluminum-virus precipitate formation. It is to be noted that their experimental conditions were entirely different and that they attempted to separate the first-stage reaction (aluminum-virus complex formation) from the second-stage (precipitation and flocculation) by assuming that the second-stage reaction would not start in the absence of SiO_2 which was found to be required for the formation of good flocs under their experimental conditions. Time required for the first-stage reaction was assumed to be the longest contact time between aluminum and virus particles at which there were no significant differences in percentages of recovery of the virus between the tests performed in the presence of SiO_2 and those in the absence of SiO_2.

It is also seen from Figure 8 that the amount of aluminum adsorbed by the MS2 particles does not change appreciably with pH in the range 5.0 to 9.0 and the variations are well within the limits of experimental error. However, the variations are more pronounced in the case of the T4 particles (Figure 7). It is not possible to provide a completely satisfactory explanation based upon the ionization of the charged groups on the coat protein of the bacteriophage T4. Thus, the differences may be due to the variations in the amount of aluminum adsorbed on the surfaces of the glassware.

(2) Quantitative Adsorption

Figures 9 and 10 show the quantitative adsorption of aluminum by bacteriophages T4 and MS2, respectively. For studies with bacteriophage T4, three different virus stock suspensions and two different aluminum concentrations were used. The three virus stocks were prepared at different times and were stored for varying periods of time ranging from 1 to 6 months at $4^{\circ}C$.

These figures show that a linear relationship exists between virus concentration and the amount of aluminum adsorbed. In other words, aluminum is reacting stoichiometrically with the virus particles. It can also be concluded from observations with bacteriophage T4 that the amount of aluminum adsorbed is not affected by the period and conditions of storage of the virus particles used. The amount of aluminum available also does not exert an effect providing the amount available is greater than the limiting concentration.

To substantiate the reliability of the Millipore membrane technique used so far in these studies, a comparison was made between this method and another method involving high speed centrifugation. Virus particles along with adsorbed aluminum were separated from the reaction mixture by centrifuging at 34,800 x g for one hr. The difference in aluminum concentrations in supernatants of the control and the reaction mixture (Table 7) was used to

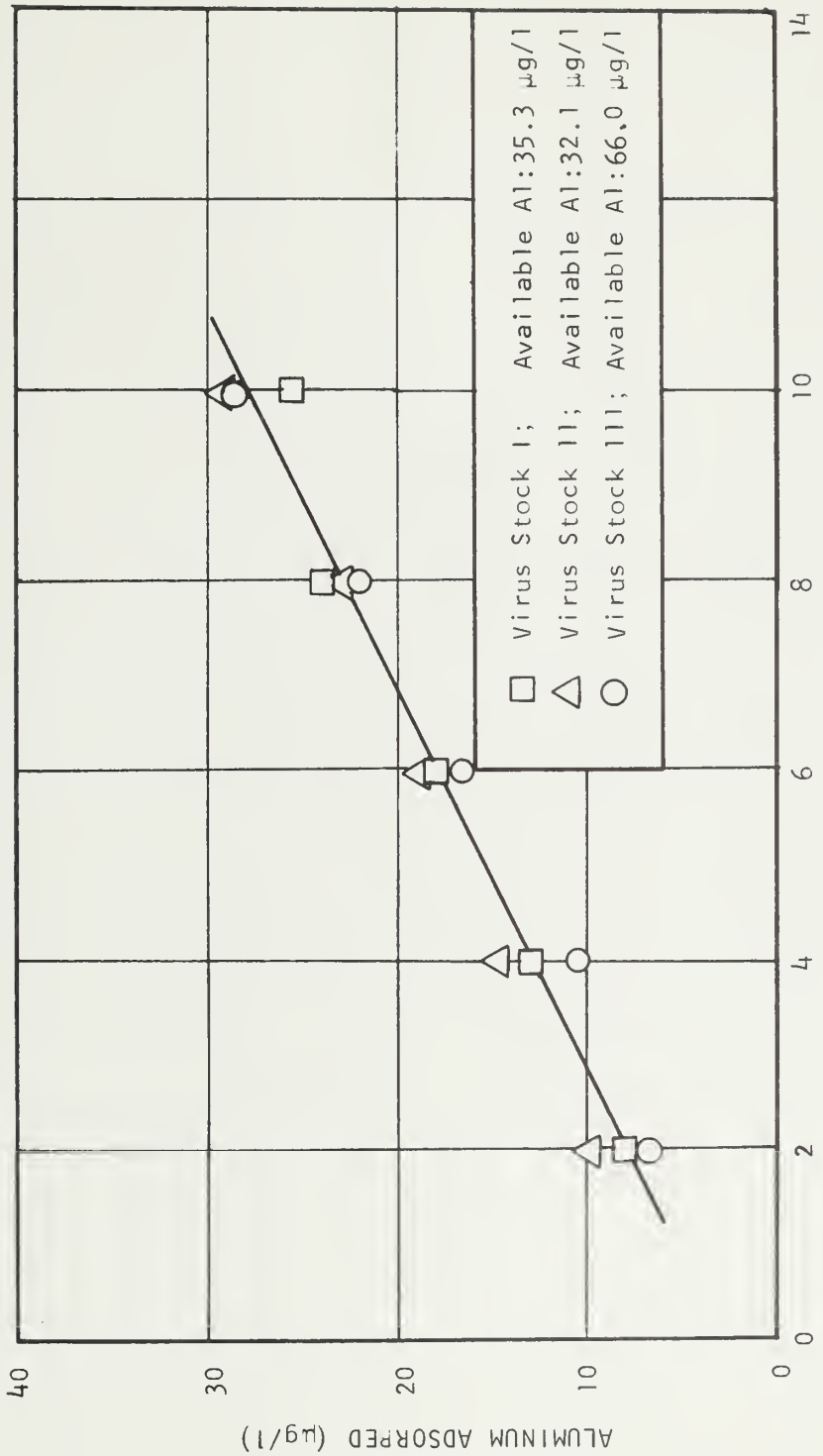

FIGURE 9. QUANTITATIVE ADSORPTION OF ALUMINUM BY BACTERIOPHAGE T4 AT pH 5.0.

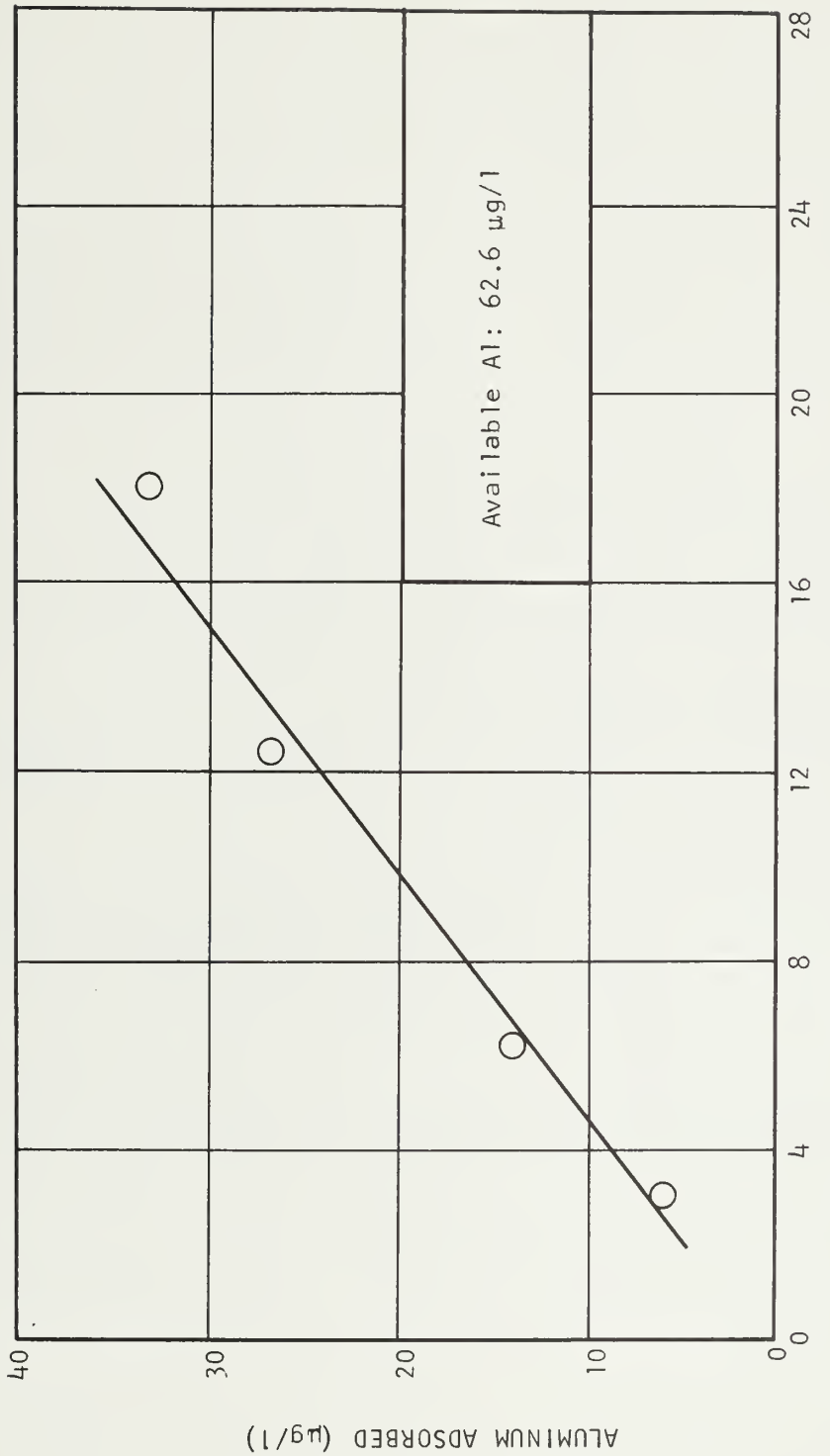

FIGURE 10. QUANTITATIVE ADSORPTION OF ALUMINUM BY BACTERIOPHAGE MS2 AT pH 5.0.

compute the quantity of aluminum adsorbed. Results agreed very well. For a bacteriophage T4 concentration of 7×10^{10}/ml, the amount of aluminum adsorbed was 20 μg/l. Using the phosphate modification method (Shull and Guthan, 1967) concentration of aluminum in the sedimented pellet was also determined for a mass balance on aluminum. The pellet was eluted with 0.2 M tris buffer (pH 8.0), digested in 1 N sulfuric acid at 15 psi for 30 min, neutralized to pH 4.5 with 1 N sodium hydroxide and the concentration of aluminum determined (Table 8).

TABLE 8

MASS BALANCE ON ALUMINUM BY CENTRIFUGATION METHOD

Virus concentration = 7×10^{10}/ml; pH = 5.0 (0.2 M Acetate Buffer)

Initial concentration of aluminum in the control and in the reaction mixture = 37.5 μg/l

Control

Concentration of aluminum in the control after centrifugation = 27.5 μg/l

$$\text{Percent loss} = \frac{37.5 - 27.5}{37.5} \times 100 = 26.7$$

Reaction Mixture

Concentration of aluminum in the supernatant of the reaction mixture after centrifugation = 7.5 μg/l

Concentration of aluminum in the sedimented pellet = 22.5 μg/l

Total aluminum recovered = 7.5 + 22.5 = 30.0 μg/l

In this example (Table 8), the initial concentration of aluminum in the control and in the reaction mixture was 37.5 μg/l. The concentration of aluminum in the control after centrifugation was 27.5 μg/l. This was the amount of aluminum available for reaction with the T4 particles. The 26.7

percent loss is assumed to be due to adsorption on the glassware and on the wall of the centrifuge tube. A total recovery of 30.0 μg/l of aluminum was made from the supernatant and the sedimented pellet from the reaction mixture. This is within 9 percent of the amount of aluminum available (27.5 μg/l). Hence excellent mass balance was obtained.

In order to further investigate the quantitative nature of aluminum adsorption, saturation curves for aluminum adsorption for different bacteriophage T4 concentrations at pH values 5.0, 6.0, and 9.0 were obtained. These curves are shown in Figures 11, 12, and 13.

The saturation curves follow the same pattern at pH values 5.0, 6.0, and 9.0. It can be seen that when the concentration of available aluminum is less than the limiting concentration, almost complete adsorption of aluminum from the solution takes place. The plateau regions of the saturation curves indicate complete saturation of the adsorption sites on the virus coat protein. Consequently, the quantity of aluminum that can be adsorbed on the virus coat protein surface is constant.

These adsorption data were then plotted according to the linear form of the Langmuir adsorption equation. This was done using the least squares method of curvefitting to facilitate the calculation of the amount of aluminum adsorbed by a T4 particle at different pH values. The basic assumptions involved are that (1) all adsorbent sites are identical and that (2) no interaction takes place between molecules adsorbed on adjacent sites (Graham, 1959). The Langmuir equation can be expressed by

$$C/q = \frac{1}{KZ} + \frac{C}{Z}$$

where

C = concentration of aluminum in the solution at equilibrium, μg/l

q = aluminum adsorbed per unit of adsorbent at equilibrium, μg/particle

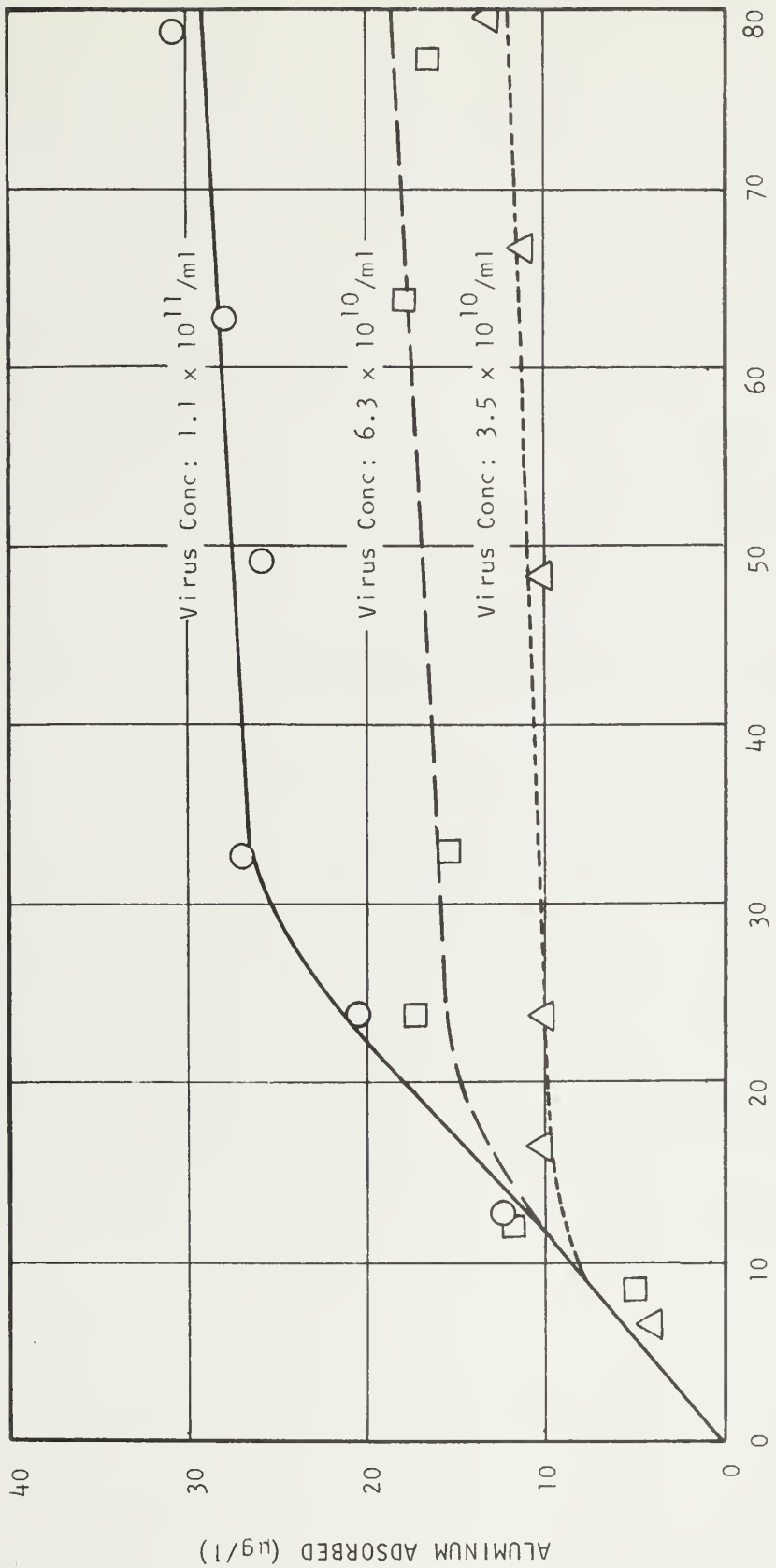

FIGURE 11. SATURATION CURVES FOR ALUMINUM ADSORPTION BY BACTERIOPHAGE T4 AT pH 5.0.

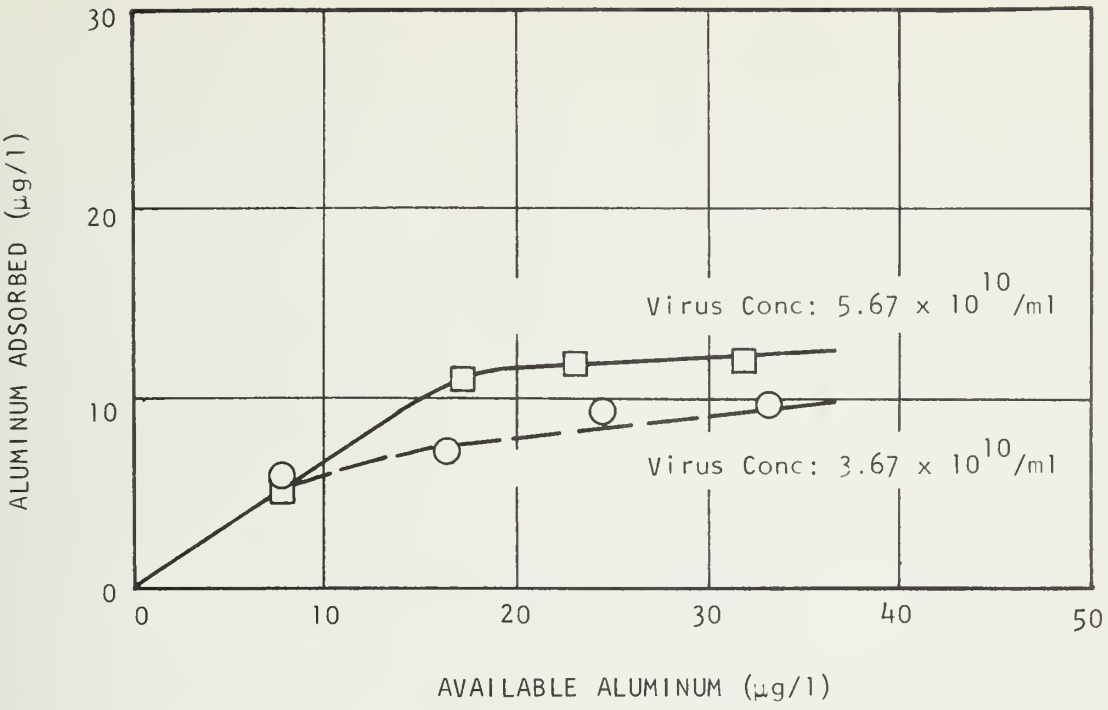

FIGURE 12. SATURATION CURVES FOR ALUMINUM ADSORPTION BY BACTERIOPHAGE T4 AT pH 6.0.

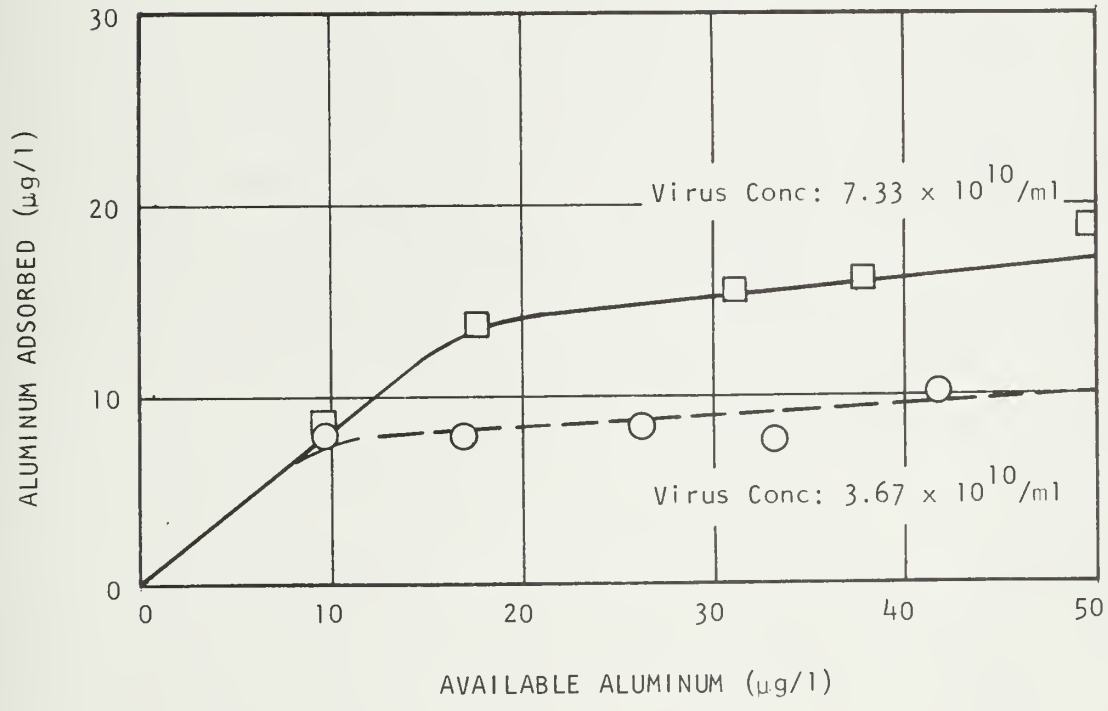

FIGURE 13. SATURATION CURVES FOR ALUMINUM ADSORPTION BY BACTERIOPHAGE T4 AT pH 9.0.

Z = saturation ratio, μg/particle

K = constant, particle/l

Adsorption data of Figures 11, 12, and 13 fit well to the linear form of the isotherm (Figures 14, 15, and 16). The amounts of aluminum adsorbed by a T4 particle at different pH values were calculated from these plots. These data are given in Table 9.

TABLE 9

AMOUNTS OF ALUMINUM ADSORBED BY A BACTERIOPHAGE T4 PARTICLE AT DIFFERENT pH VALUES

pH	Aluminum adsorbed	
	μg/particle	atoms/particle
5.0	3.31×10^{-13}	7.37×10^{3}
6.0	2.79×10^{-13}	6.21×10^{3}
9.0	2.83×10^{-13}	6.30×10^{3}

Even though saturation curves were not obtained for the bacteriophage MS2 - aluminum system, an estimate can be made of the amount of aluminum adsorbed by a MS2 particle at pH 5.0. Taking values from Figure 10, this appears to be 2.05×10^{-13} μg/particle or 4.6×10^{3} atoms/particle. Due to the smaller size of a MS2 particle compared to a T4 particle, lesser amount of aluminum is adsorbed.

It is evident that the amounts of aluminum adsorbed by a T4 particle at pH values 6.0 and 9.0 are same and the variations reported are well within the limits of experimental error. However, it is not possible to provide a completely satisfactory explanation for the difference in the amounts of aluminum

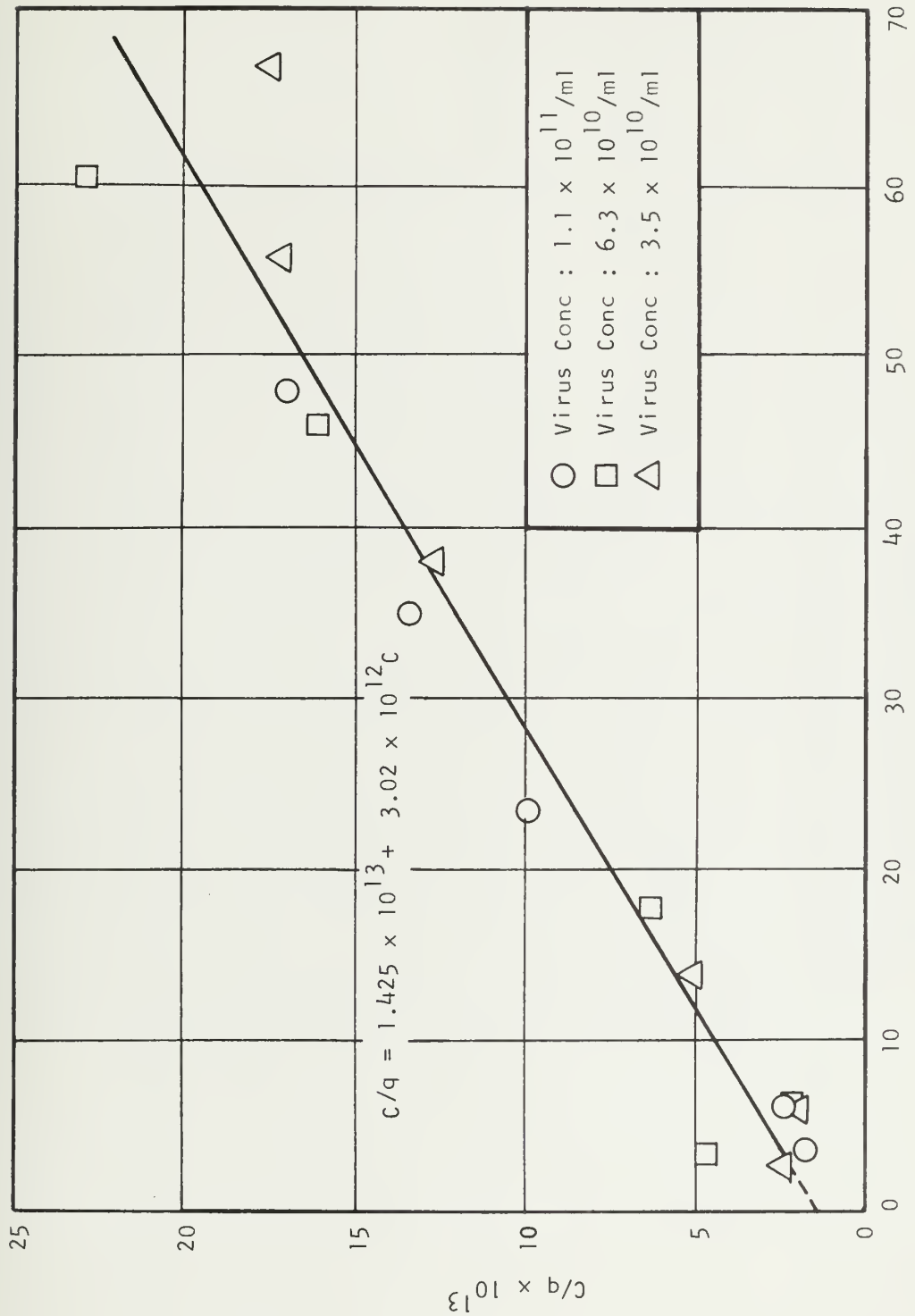

FIGURE 14. McBAIN - BRITTON PLOT OF ADSORPTION DATA OF BACTERIOPHAGE T4 - ALUMINUM SYSTEM (pH 5.0) TO FIT LANGMUIR ADSORPTION EQUATION.

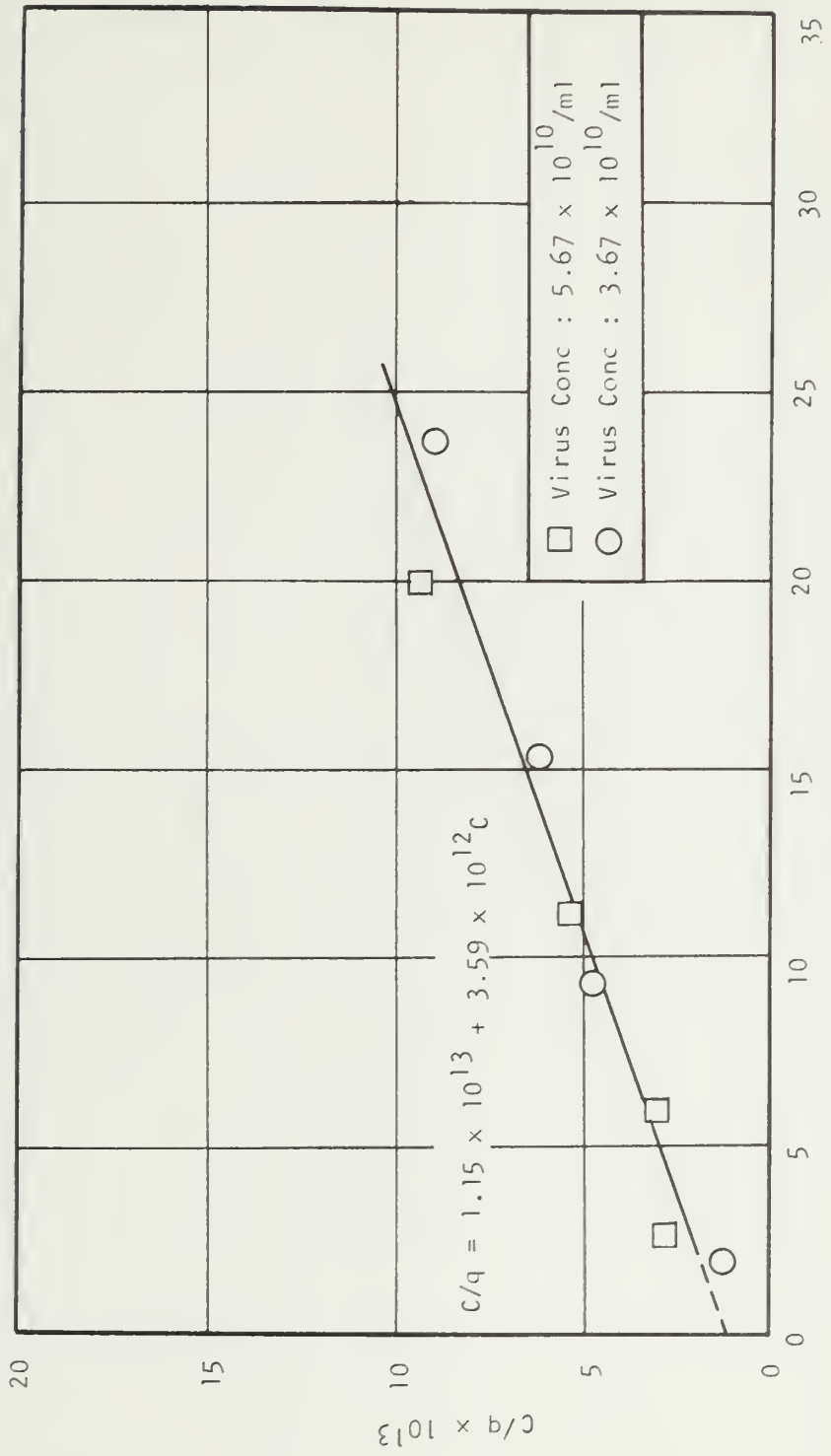

FIGURE 15. McBAIN - BRITTON PLOT OF ADSORPTION DATA OF BACTERIOPAGE T4 - ALUMINUM SYSTEM (pH 6.0) TO FIT LANGMUIR ADSORPTION EQUATION.

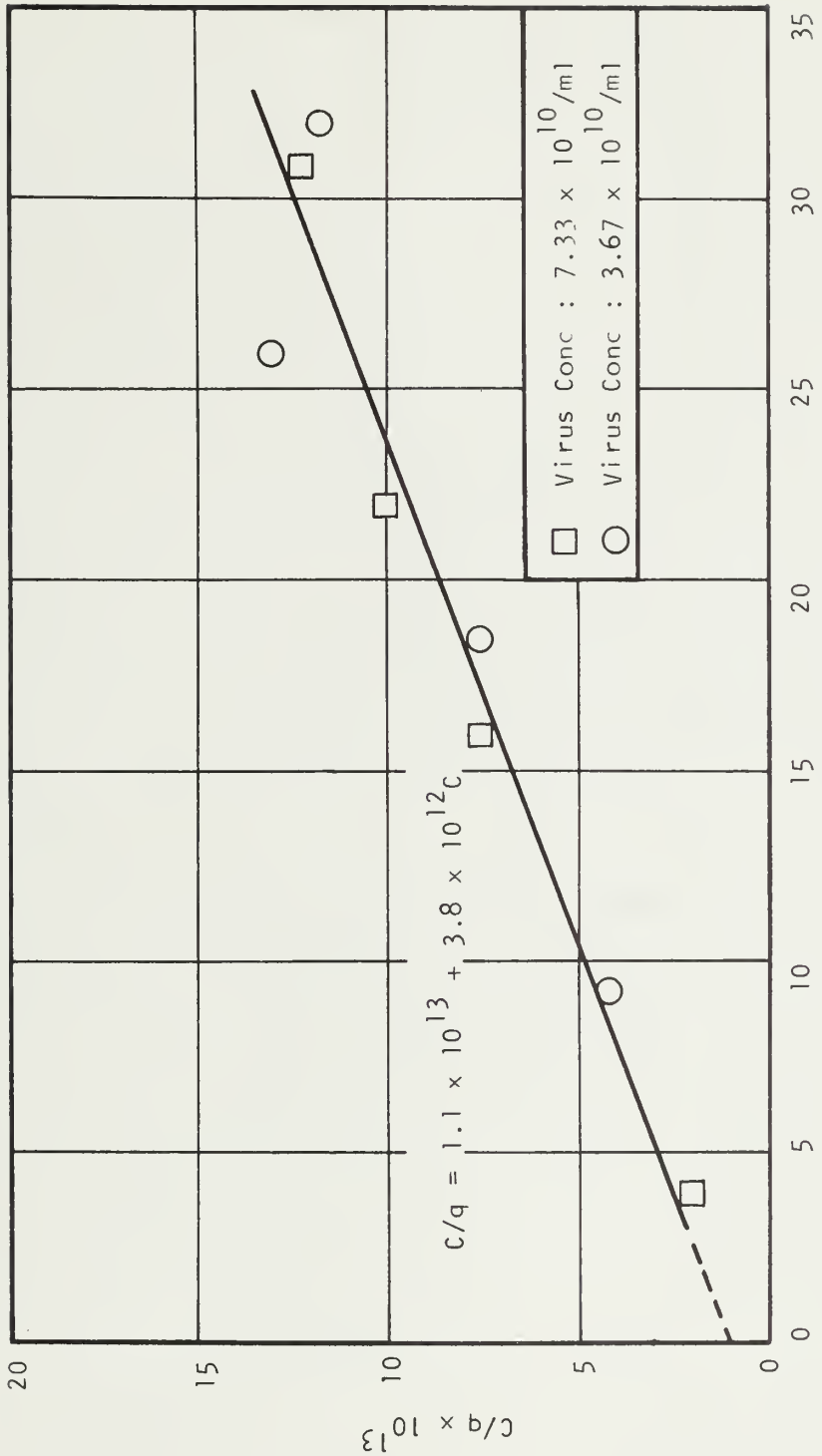

FIGURE 16. McBAIN - BRITTON PLOT OF ADSORPTION DATA OF BACTERIOPHAGE T4 - ALUMINUM SYSTEM (pH 9.0) TO FIT LANGMUIR ADSORPTION EQUATION.

adsorbed at pH values 5.0 and 6.0. The ionization of the charged groups on the bacteriophage T4 coat protein may not be appreciably different at these two pH values. The fact that a stoichiometric amount of aluminum is adsorbed by a T4 particle at pH values between 5.0 and 9.0 leads to the likelihood of a reaction of the "complex formation" type. Coordination complexes between aluminum and some ionic group(s) on the coat protein of bacteriophage is quite likely. Physical adsorption involving electrostatic attraction is ruled out because of the excellent stoichiometry between aluminum and T4 particles in spite of the fact that the predominant aqueous aluminum species at pH values 5.0 and 6.0 are positively charged whereas the species at pH 9.0 is negatively charged (Figure 1). The positively charged species are either $Al_7(OH)_{17}^{+4}$ and $Al_{13}(OH)_{34}^{+5}$ or $Al_6(OH)_{15}^{+3}$ and $Al_8(OH)_{20}^{+4}$. The negatively charged species is $Al(OH)_4^-$. It seems quite likely that aluminum bound in these hydroxo complexes is complexing with the ionic group(s) on the coat protein of the bacteriophage.

By considering the protein coat of bacteriophage T4, it is possible to make some approximate calculations as to the availability of ionic groups for the formation of coordination complexes with aluminum. Detail amino acid composition of the T4 coat protein is available in the literature (Table 10). The head of the phage particle is made up of about 300 identical protein subunits (Edgar and Epstein, 1965), which is the major protein constituent of the phage (Stent, 1963). A large protein generally consists of subunits which are the minimal physical units. Assuming about 200 amino acid units in a protein subunit, the total number of amino acid units in the head protein are about 6×10^4. This estimate and the mean amino acid composition of the T4 coat protein lead to the following approximate calculations.

TABLE 10

AMINO ACID COMPOSITION OF BACTERIOPHAGE T4

Amino Acid	Mole Percent		
	Fitch and Susman (1965)	Polson and Wyckoff (1948)	Mean
Lysin	6.4	7.1	6.75
Histidine	0.0	2.0	1.00
Arginine	4.1	4.5	4.30
Aspartate and Aspargine	11.1	11.1	11.10
Threonine	7.0	7.1	7.05
Serine	5.9	5.5	5.70
Glutamate and glutamine	10.4	9.9	10.15
Proline	4.1	5.3	4.70
Glycine	9.4	11.8	10.60
Alanine	11.8	12.8	12.30
Valine	6.9	6.8	6.85
Methionine	2.1	1.1	1.60
Isoleucine	6.5	3.6	5.05
Leucine	6.0	6.0	6.00
Tyrosine	4.1	2.5	3.30
Phenylalanine	4.3	3.1	3.70
Amide Content	46.6*	-	-

*Mole percent of total dicarboxylic acids.

Number of carboxyl groups

= (Number of terminal carboxyl groups) + (Total number of ω-carboxyl groups due to aspartate and glutamate residues - number of ω-carboxyl groups combined with ammonia in amide linkage)

$$= 300 + \left\{(11.10 + 10.15) \times \frac{(100 - 46.6)}{100}\right\} \times \frac{6 \times 10^4}{100}$$

= 7,120

Number of hydroxyl groups

= Number of phenolic hydroxyl groups of tyrosine

$$= \frac{3.30}{100} \times 6 \times 10^4$$

= 1,980

Number of ammonium and guanidinium groups

= (Number of terminal ammonium groups) + (Number of ε-ammonium groups of Lysine) + (Number of guanidinium groups of arginine)

$$= 300 + \frac{(6.75 \times 6 \times 10^4)}{100} + \frac{4.3 \times 6 \times 10^4}{100}$$

= 6,930

The amount of aluminum adsorbed by a T4 particle is in the range of 6,210 to 7,370 atoms of aluminum (Table 9). From a comparison of these calculations and the data in Table 9, it seems quite possible from a consideration of the head protein only that adsorption of aluminum is due to the formation of coordination complexes between aluminum and either the carboxyl or ammonium groups. However, it is more probable that the carboxyl groups are involved because these groups have been postulated to be responsible for metal ion binding by proteins (Fruton and Simmonds, 1953, and Stumm and Morgan, 1962). On the basis of pH-metric and viscometric observations, carboxyl groups have also been shown to be involved in binding of aluminum by casein, gelatin, etc. (Schulman and Dogan, 1952; Salahuddin and Malik, 1962, 1964; Salahuddin, 1964; and Malik and Salahuddin, 1965).

B. Virus Inactivation by Aluminum

In order to study the condition of the "complexed" viruses, inactivation of the bacteriophages was studied in the presence of soluble aluminum. Figures 17 and 18 show the inactivation of bacteriophages T4 and MS2, respectively, at pH 5.0 in the presence of soluble aluminum. Inactivation of the bacteriophages at pH 6.0 and 9.0 was not studied because it was thought that the inactivation studies at pH 5.0 would reveal the entire picture due to the fact that the kinetics and the quantity of adsorption of aluminum by these bacteriophages are identical at pH values 5.0, 6.0, and 9.0.

It is seen that inactivation rates of both the bacteriophages in the presence of various soluble aluminum concentrations are the same as those of the control without any aluminum. Since bacteriophage assay involves adsorption of bacteriophage on the surface of the host bacterium, it may be concluded that different sets of sites or ionic groups are involved in the electrostatic interaction between the bacteriophage and the host bacterium preceding an infection and in the interaction between aluminum and the bacteriophage. Furthermore, complexing of aluminum does not in any manner interfere with the capacity of the phage particle to infect its host.

From the preceding discussion it is quite logical to assume that virus particles removed from a water supply by chemical coagulation and flocculation could remain "viable" in the settled sludge. In order to study this aspect, attempts were made to recover the bacteriophage particles from the settled floc following their removal from water by chemical coagulation and flocculation (jar tests). Table 11 shows the extent to which bacteriophages T4 and MS2 could be recovered from the settled floc by different elutants. Recoveries greater than 53.75 percent were never obtained. This is presumably due to the fact that the elutants used were not able to completely dissociate the

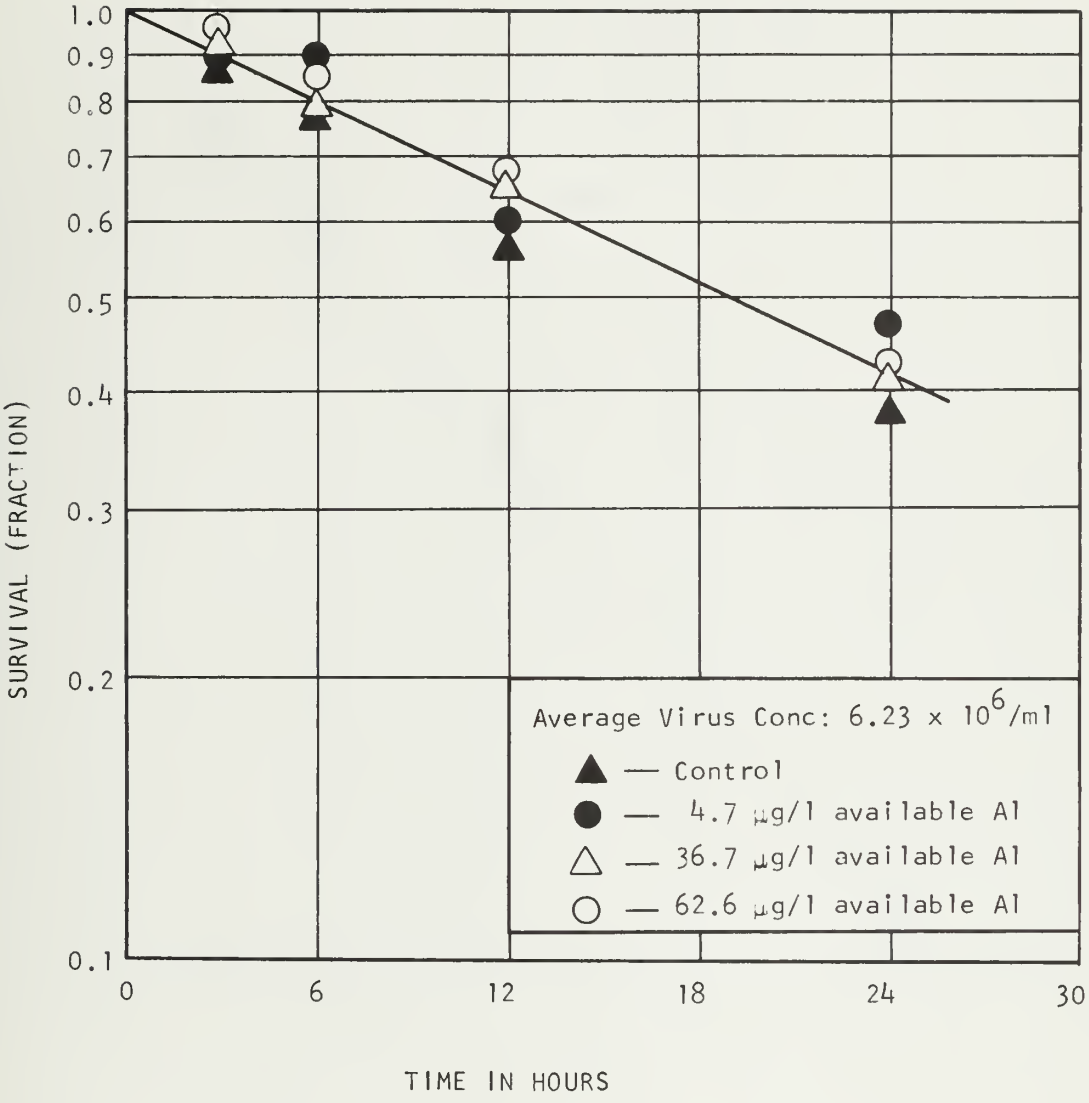

FIGURE 17. INACTIVATION OF BACTERIOPHAGE T4 IN THE PRESENCE OF SOLUBLE ALUMINUM AT pH 5.0.

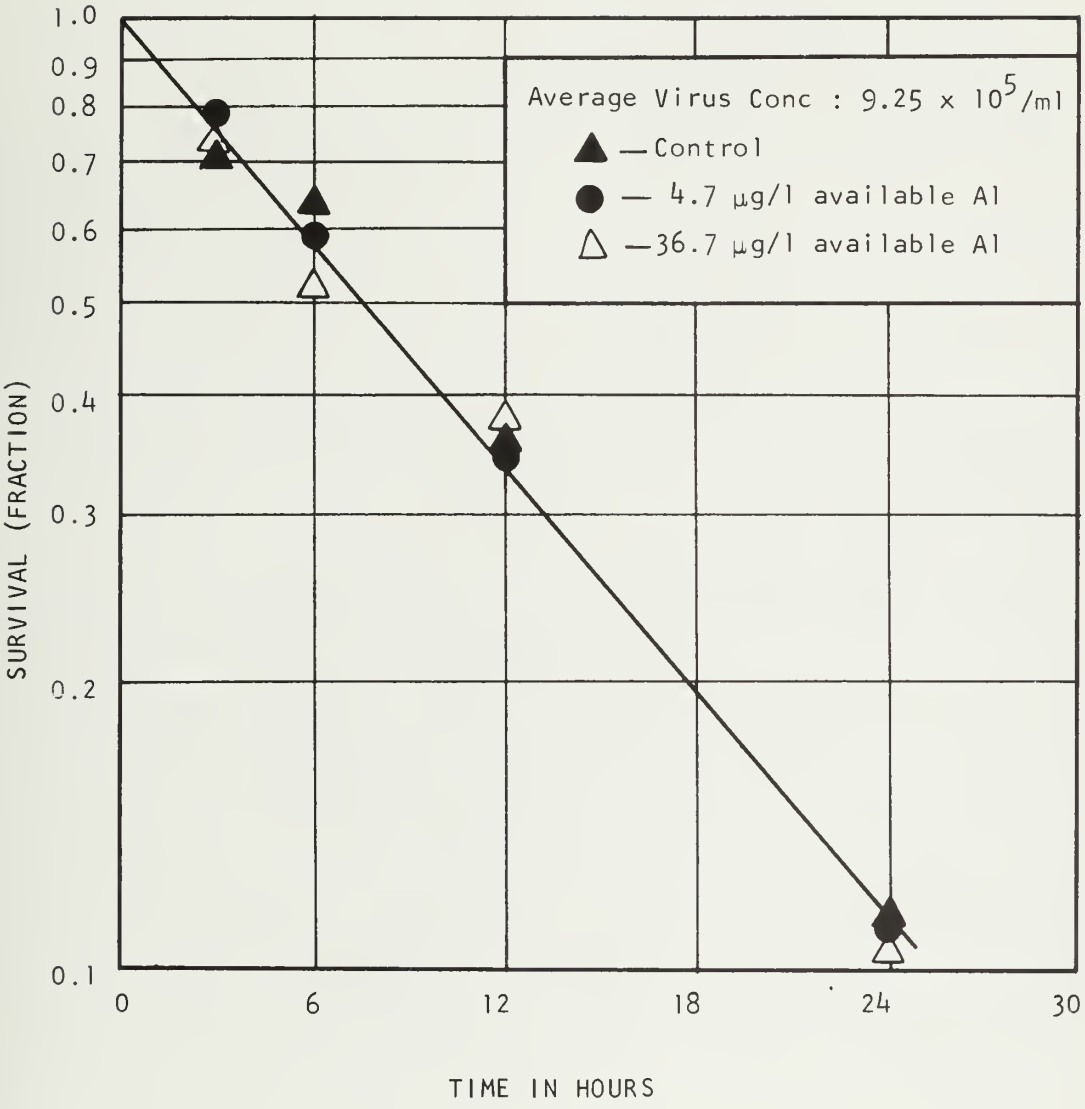

FIGURE 18. INACTIVATION OF BACTERIOPHAGE MS_2 IN THE PRESENCE OF SOLUBLE ALUMINUM AT pH 5.0.

TABLE 11

RECOVERY OF BACTERIOPHAGES FROM SETTLED FLOC FOLLOWING CHEMICAL COAGULATION AND FLOCCULATION

Elutant	Percent Recovered	
	Bacteriophage T4	Bacteriophage MS2
Deionized Water	28.50	-
3% Beef Extract	52.27	52.85
1% Bovine Serum Albumine	53.75	-
0.1 M Tris Buffer (pH 8.0)	42.05	-
0.2 M Phosphate Buffer (pH 8.0)	42.95	45.25

bacteriophage particles from the floc resulting in infection of a host bacterial cell by more than one bacteriophage.

Chang, Isaac and Baine (1953) were able to recover 60 percent of the removed viruses by redispersing the flocculated mixture at pH 7.6 with vigorous stirring. It is evident from these observations that disposal of water treatment plant sludges treating water containing pathogenic viruses might constitute a public health hazard. Especially in the case of land disposal of water treatment plant sludges, this might constitute a ground water contamination problem.

C. Virus Removal by Chemical Coagulation and Flocculation (Jar Tests)

(1) Optimum pH and Aluminum Sulfate Dosages

In presenting the results of the coagulation and flocculation studies (jar tests), virus removals are reported as percent removals. It should be noted that for an initial input virus concentration of 4×10^5/ml, a removal of 99 percent would mean a reduction in the virus titer from 4×10^5/ml to

4×10^3/ml in the supernatant. Correspondingly a removal of 98 percent would mean a reduction in the virus titer from 4×10^5/ml to 8×10^3/ml and so on.

Figure 19 shows the removal of bacteriophage T4 and clay turbidity at different pH values and coagulant dosages. It is interesting to note that virus removal closely parallels turbidity removal. It can be seen from this figure that based on both virus and turbidity removal, the optimum aluminum sulfate dosage and pH for bacteriophage T4 removal was 40 to 50 mg/l (1.2×10^{-4} M to 1.5×10^{-4} M as aluminum) and the lowest pH studied (5.24). The highest removal obtained was 98 percent. All subsequent experiments with bacteriophage T4 were performed at pH values ranging from 5.2 to 5.3 with 50 mg/l of aluminum sulfate.

Removal of bacteriophage MS2 is shown in Figure 20. The optimum aluminum sulfate dosage and pH was 40 to 50 mg/l (1.2×10^{-4} M to 1.5×10^{-4} M as aluminum) at pH 6.0. Removal was 99.9 percent. Turbidity removal curves for MS2 were similar to those shown in Figure 19. Removals higher than 99.3 percent were never obtained at pH 5.10 even with higher aluminum sulfate dosage. However, all subsequent experiments with bacteriophage MS2 were performed at pH 6.0 with 50 mg/l of aluminum sulfate.

(2) Effect of Calcium and Magnesium on Virus Removal

Chang et al. (1958b) believed that the presence of calcium and magnesium ions in raw water interfered with virus removal by reducing the rate of coagulant-cation bacterial-virus complex formation. Experiments were performed to investigate this phenomenon in further detail. Figure 21 shows the kinetics of adsorption of aluminum by bacteriophage T4 at pH values 5.0 and 9.0 in the presence of 50 mg/l of each of the cations calcium and magnesium. Effect of these two cations on the removal of bacteriophage T4 by

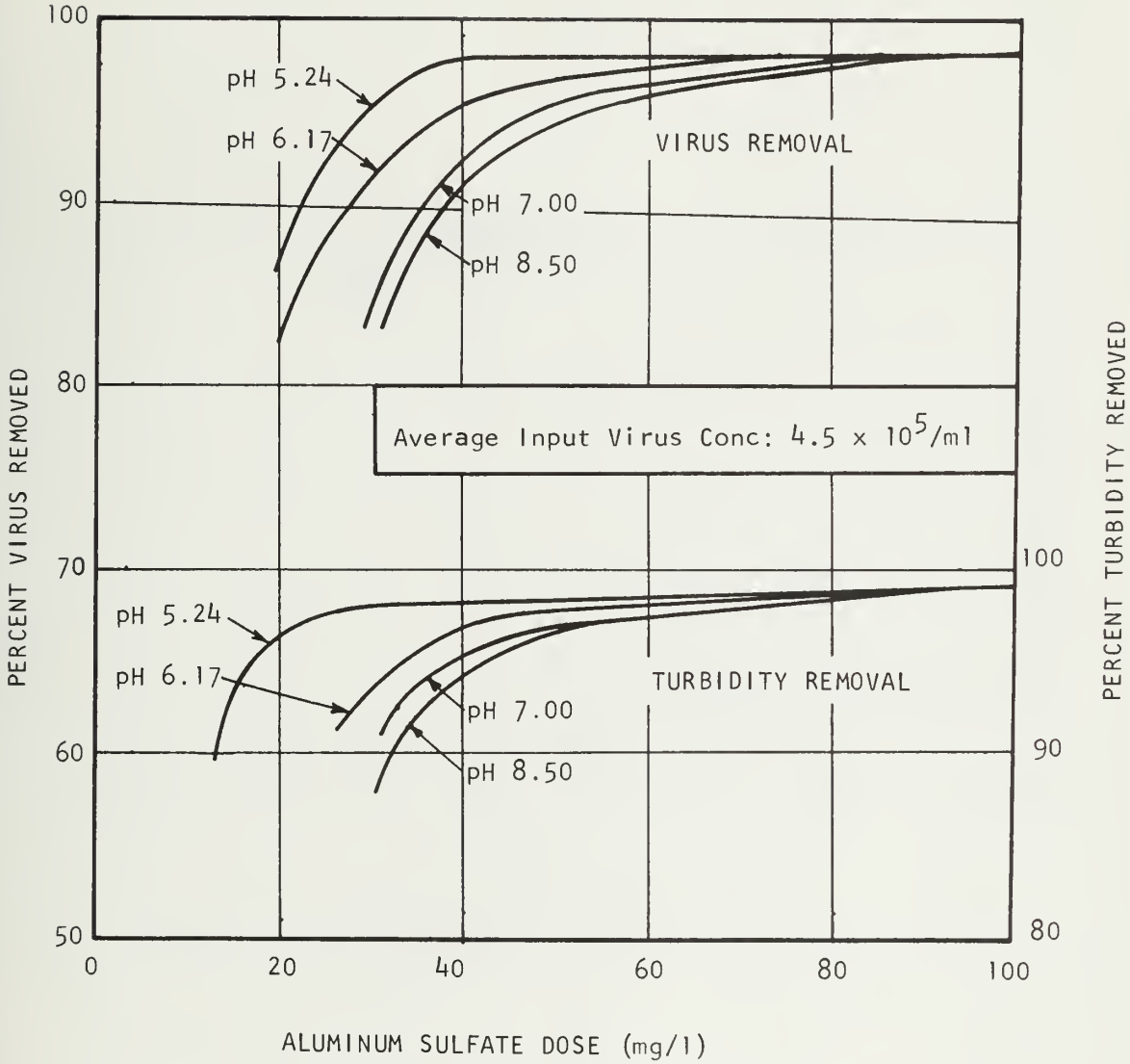

FIGURE 19. REMOVAL OF BACTERIOPHAGE T4 AND CLAY TURBIDITY BY COAGULATION AND FLOCCULATION.

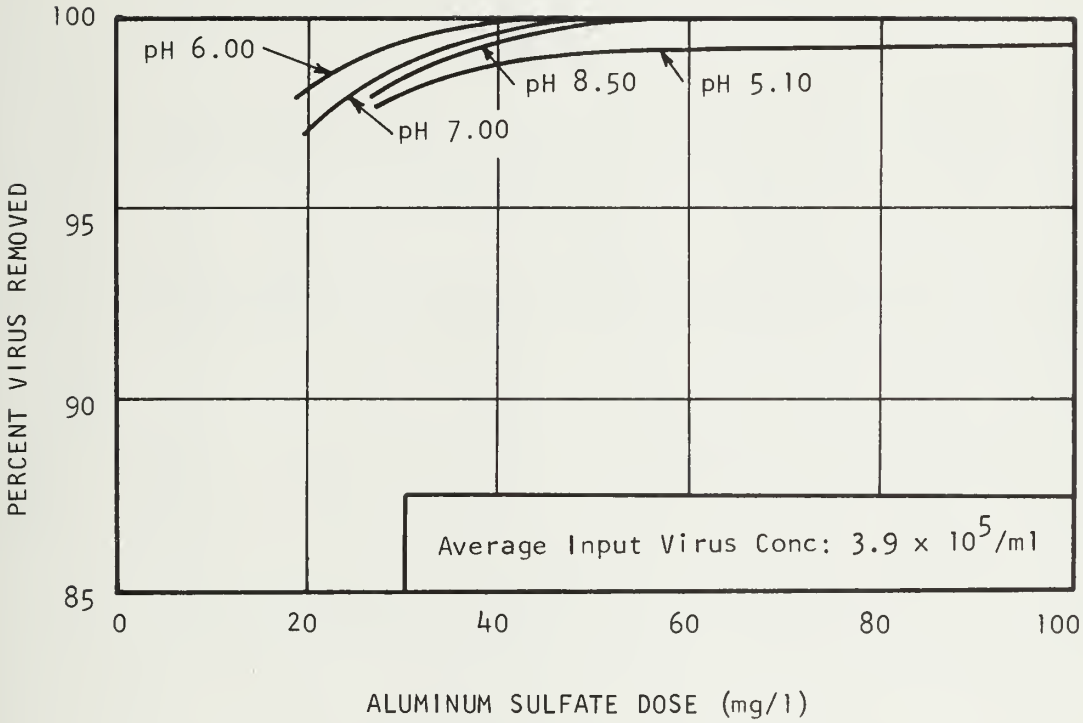

FIGURE 20. REMOVAL OF BACTERIOPHAGE MS_2 BY COAGULATION AND FLOCCULATION.

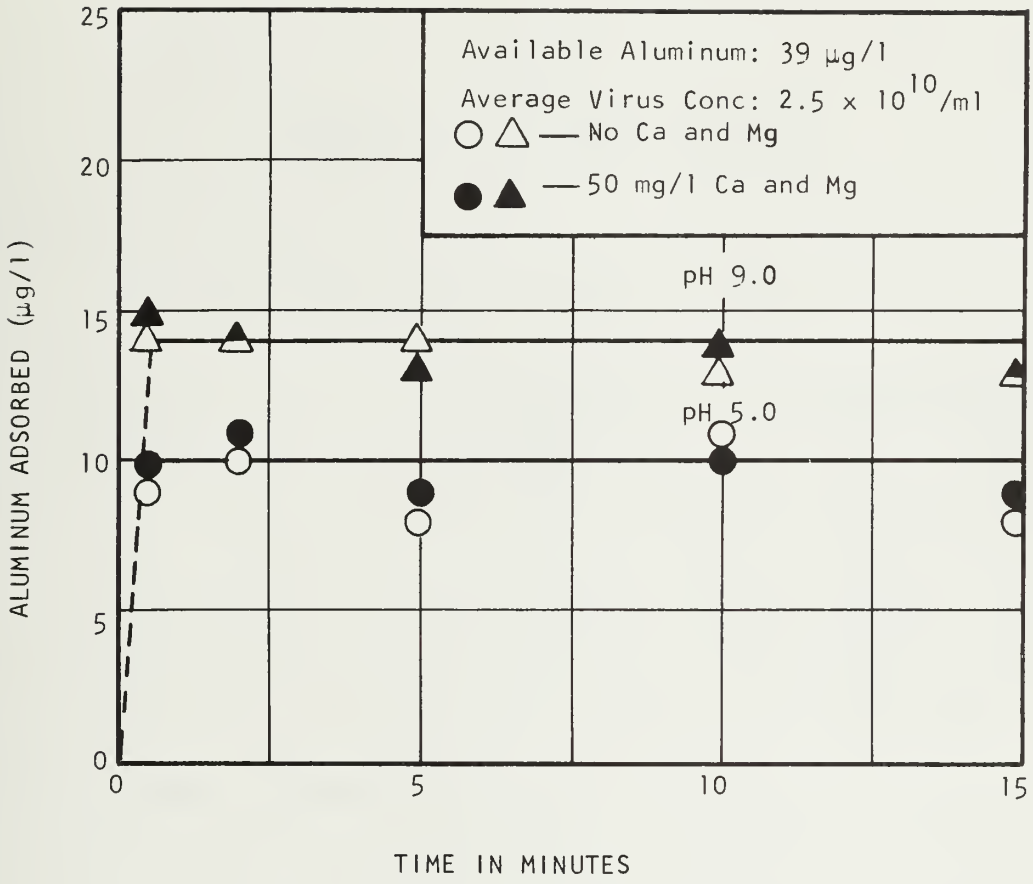

FIGURE 21. KINETICS OF ADSORPTION OF ALUMINUM BY BACTERIOPHAGE T4 IN THE PRESENCE OF CALCIUM AND MAGNESIUM.

coagulation and flocculation (jar tests) is shown in Table 12. It was not possible to conduct jar tests at pH 9.0 due to the limitation of the carbonic acid-bicarbonate buffer system, the upper limit of pH for this system being 8.3.

TABLE 12

REMOVAL OF BACTERIOPHAGE T4 IN THE PRESENCE OF CALCIUM AND MAGNESIUM

Average Input Virus Concentration: 4.05×10^5/ml; pH range: 5.1 - 5.4

Aluminum Sulfate: 50 mg/l; Average Turbidity: 12.5 JTU

Calcium mg/l	Magnesium mg/l	Percent Removal
0	0	97.86
25	25	98.02
50	50	97.91

It is evident that presence of calcium and magnesium ions up to a concentration of 50 mg/l does not interfere either with kinetics of adsorption of aluminum or with removal of bacteriophage T4 by coagulation and flocculation. The fact that presence of bivalent cations like calcium and magnesium does not change the kinetics and stoichiometry of aluminum virus interaction is further evidence for the formation of coordination complexes between aluminum and virus coat protein.

(3) Effect of Organic Matter on Virus Removal

It is logical to assume that the presence of extraneous organic matter of proteinaceous character will interfere with virus removal by coagulation and flocculation. This may occur because of the competitive action of the organic

matter in question with the virus particles in the coagulation and flocculation reaction. To study this effect, virus removal in the presence of albumin, wastewater and wastewater effluent was studied. Table 13 shows the removal of bacteriophage T4 in the presence of egg albumine, bovine serum albumin, settled wastewater and wastewater effluent. Removal of bacteriophage MS2 in the presence of settled wastewater and wastewater effluent is shown in Table 14.

It is seen that egg albumin, bovine serum albumin and settled wastewater interfered with bacteriophage T4 removal in the pH range 5.1-5.4 (Table 13). The albumins also interfered with the flocculation process as evidenced by lower turbidity removals. The effect of wastewater effluent was not very much pronounced either on turbidity removal or on the removal of bacteriophage T4 in the pH range 5.1 - 5.4. However, considerably lower removals were obtained for turbidity and bacteriophage MS2 in the pH range 5.9-6.1 in the presence of settled wastewater or wastewater effluent (Table 14). These observations indicate that the process of coagulation and flocculation may not be expected to operate with high efficiency if the raw water contains organic matter.

(4) Effect of Preformed Floc on Virus Removal

At this stage it is clear from the discussions in the preceding sections that the nature of the interaction between aluminum and virus is most probably that of a coordination complex formation. Consequently it follows that intimate contact between the virus particles and the soluble aluminum species is necessary before the formation of any hydrated aluminum oxide precipitate in a coagulation and flocculation system. Experiments were performed to study this aspect by adding the virus stock suspension to the water at various times after the addition of aluminum sulfate. Table 15 shows the results of this study.

TABLE 13

REMOVAL OF BACTERIOPHAGE T4 BY COAGULATION AND FLOCCULATION IN THE PRESENCE OF ORGANIC MATTER

Average Input Virus Concentration: 3.58×10^5/ml; pH range: 5.1 - 5.4

Aluminum Sulfate: 50 mg/l; Average Turbidity; 12.5 JTU*

Organic Matter	Concentration	Percent Removal	
		Bacteriophage T4	Turbidity
Egg Albumin	0 mg/l	98.02	99.00
	10 "	96.89	98.24
	20 "	96.38	98.20
	30 "	95.29	98.08
	50 "	94.83	95.84
Bovine Serum Albumin	0 mg/l	98.65	99.12
	10 "	97.67	98.80
	20 "	97.63	98.60
	30 "	96.85	98.40
	50 "	96.00	95.44
Settled Wastewater**	0 ml/l	97.40	98.37
	200 "	95.71	98.63
Wastewater Effluent**	0 ml/l	97.40	98.37
	200 "	97.03	98.77

*Initial turbidity values ranged from 18 to 32 JTU when settled wastewater was added.

**Characteristics:

	Raw Wastewater	Wastewater Effluent
5-day BOD (mg/l)	445	21
Total suspended solids (mg/l)	210	37
Volatile suspended solids (mg/l)	194	11

TABLE 14

REMOVAL OF BACTERIOPHAGE MS2 BY COAGULATION AND FLOCCULATION IN THE PRESENCE OF WASTEWATER AND WASTEWATER EFFLUENT

Average Input Virus Concentration: 3.87×10^5/ml; pH range: 5.9 - 6.1

Aluminum Sulfate: 50 mg/l; Average Turbidity: 12.5 JTU*

	Percent Removal	
	Bacteriophage MS2	Turbidity
No Settled Wastewater or Wastewater Effluent	99.82	98.10
200 ml/l Settled Wastewater**	89.80	92.10
200 ml/l Wastewater Effluent**	94.00	93.60

*Initial turbidity was 19.0 JTU when settled wastewater was added.

**Characteristics:

	Raw Wastewater	Wastewater Effluent
5-day BOD (mg/l)	181	50
Total suspended solids (mg/l)	253	10
Volatile suspended solids (mg/l)	178	9

TABLE 15

REMOVAL OF BACTERIOPHAGE T4 BY PREFORMED FLOCS

Average Input Virus Concentration: 4.0×10^5/ml; pH range: 5.1 - 5.4

Aluminum Sulfate: 50 mg/l; Average Turbidity: 12.5 JTU

Bacteriophage T4 added (minutes after aluminum sulfate addition)	Percent Removal		Remarks
	Bacteriophage T4	Turbidity	
-	98.04	99.17	Control
-	80.60	97.0	Preformed floc not in situ. Bacteriophage T4 added one min after addition of preformed floc.
1	76.40	99.0	
5	60.50	99.04	
15	37.25	99.17	

It can be seen that preformed floc was not very effective in removing bacteriophage T4 from the water. Similar observations also have been reported in the literature (Chang, Isaac, and Baine, 1953). These observations demonstrate that intimate contact between the virus particles and aluminum is necessary before their incorporation into the floc masses of hydrated aluminum oxide precipitates and subsequent removal by settling. In contrast, removal of clay turbidity by coagulation and flocculation with aluminum sulfate is due to a physical interaction between aluminum and the clay particles resulting in adsorption of polynuclear aluminum hydrolysis species to the clay particles and consequent aggregation by interparticle bridging involving particle transport and chemical interaction (Stumm and O'Melia, 1968).

(5) Effect of Polyelectrolytes on Virus Removal

Commercially available synthetic polyelectrolytes (coagulant aids) are being used extensively in the water treatment industry for better coagulation and flocculation and longer filter runs. It was thought appropriate to study the effect of these polyelectrolytes on virus removal by chemical coagulation and flocculation (jar tests). Inactivation of bacteriophages T4 and MS2 in the presence of the polyelectrolytes in deionized water was studied in order to be able to interpret the results of the jar tests using the polyelectrolytes. Samples for bacteriophage assay were withdrawn after a contact time of 1 hr. Table 16 shows the results of this study. Tables 17 to 22 show the results of the jar tests performed at pH values of approximately 5.2 and 6.0 for bacteriophages T4 and MS2, respectively. These were the best pH values for their removal in jar tests.

It is evident from Table 16 that both the cationic polyelectrolytes, Primafloc C-7 and Catfloc, inactivated bacteriophages T4 and MS2. This was presumably due to adsorption of the phage particles to the cationic sites on

TABLE 16

INACTIVATION OF BACTERIOPHAGES T4 AND MS2
IN THE PRESENCE OF POLYELECTROLYTES (COAGULANT AIDS)

Average Input Virus Concentration: 3.44×10^5/ml

Polyelectrolyte	Concentration mg/l	pH	Bacteriophage	Percent Inactivation
Primafloc C-7	0.5-1.5	5.2	T4	81*
	0.5	6.0	MS2	85
Catfloc	0.5-10.0	5.2	T4	77*
	1.0	6.0	MS2	82
Primafloc A-10	1.0-5.0	5.2	T4	None
	1.0	6.0	MS2	None
Coagulant Aid #243	1.0-5.0	5.2	T4	None
	1.0	6.0	MS2	None

*Average value for the concentration range of the polyelectrolyte.

TABLE 17

REMOVAL OF BACTERIOPHAGE T4 BY COAGULATION AND FLOCCULATION WITH ALUMINUM SULFATE AND CATIONIC POLYELECTROLYTES AS COAGULANT AIDS

Average Input Virus Concentration: 4.49×10^5/ml; pH range: 5.1 - 5.4

Aluminum Sulfate: 50 mg/l; Average Turbidity: 12.5 JTU

Cationic Polyelectrolyte	Concentration mg/l	Percent Removal	
		Bacteriophage T4	Turbidity
	0.00	97.71	99.08
	0.50	99.82	98.87
Primafloc C-7	1.00	99.90	97.46
	1.50	99.95	95.50
	2.00	99.99	94.30
	0.00	97.84	99.32
	0.25	98.41	99.32
Catfloc	0.50	99.50	99.36
	1.00	99.96	98.98
	1.50	99.93	98.47

TABLE 18

REMOVAL OF BACTERIOPHAGE MS2 BY COAGULATION AND FLOCCULATION WITH ALUMINUM SULFATE AND CATIONIC POLYELECTROLYTES AS COAGULANT AIDS

Average Input Virus Concentrations: 2.74×10^5/ml; pH range: 5.9 - 6.0

Aluminum Sulfate: 50 mg/l; Average Turbidity: 12.5 JTU

Cationic Polyelectrolyte	Concentration mg/l	Percent Removal	
		Bacteriophage MS2	Turbidity
	0.0	99.84	98.25
	1.0	99.54	98.25
Primafloc C-7	2.0	99.23	96.58
	3.0	99.38	93.75
	0.0	99.74	98.56
	1.0	99.71	98.40
Catfloc	2.0	99.60	97.67
	3.0	98.87	96.24

TABLE 19

REMOVAL OF BACTERIOPHAGE T4 BY COAGULATION AND FLOCCULATION WITH CATIONIC POLYELECTROLYTES AS PRIME COAGULANTS

Average Input Virus Concentration: 4.98×10^5/ml

pH range: 5.2 - 5.5; Average Turbidity: 12.5 JTU

Cationic Polyelectrolyte	Concentration mg/l	Percent Removal	
		Bacteriophage T4	Turbidity
Primafloc C-7	2.5	84.40	89.10
	5.0	99.27	98.43
	7.5	99.93	98.95
	10.0	99.98	79.70
	20.0	99.99	6.50
Catfloc	1.0	17.00	0.00
	2.5	52.40	62.60
	5.0	85.70	88.70
	7.5	97.22	98.17
	10.0	99.04	98.96
	12.5	99.52	99.11
	15.0	99.84	96.75
	20.0	99.93	80.00
	25.0	99.94	72.90

TABLE 20

REMOVAL OF BACTERIOPHAGE MS2 BY COAGULATION AND FLOCCULATION WITH CATIONIC POLYELECTROLYTES AS PRIME COAGULANTS

Average Input Virus Concentration: 2.8×10^5/ml

pH range: 5.8 - 5.9; Average Turbidity: 12.5 JTU

Cationic Polyelectrolyte	Concentration mg/l	Percent Removal	
		Bacteriophage MS2	Turbidity
Primafloc C-7	2.5	93.05	89.25
	5.0	98.72	97.84
	7.5	99.15	97.84
	10.0	97.30	80.00
	20.0	95.40	15.00
Catfloc	5.0	98.02	86.65
	10.0	99.57	97.08
	12.5	99.21	96.75
	15.0	98.74	96.30
	20.0	98.76	76.70

TABLE 21

REMOVAL OF BACTERIOPHAGE T4 BY COAGULATION AND FLOCCULATION WITH ALUMINUM SULFATE AND ANIONIC POLYELECTROLYTES AS COAGULANT AIDS

Average Input Virus Concentration: 2.78×10^5/ml; pH range: 5.0 - 5.4

Aluminum Sulfate: 50 mg/l; Average Turbidity: 12.5 JTU

Anionic Polyelectrolyte	Concentration mg/l	Percent Removal	
		Bacteriophage T4	Turbidity
	0.0	97.13	99.00
	0.5	97.20	98.82
Primafloc A-10	1.0	97.20	97.91
	2.5	97.33	97.73
	5.0	97.88	96.36
	0.0	98.03	99.14
	1.0	97.80	99.14
Coagulant Aid #243	2.0	97.22	98.95
	5.0	96.93	98.18
	10.0	96.60	96.57

TABLE 22

REMOVAL OF BACTERIOPHAGE MS2 BY COAGULATION AND FLOCCULATION WITH ALUMINUM SULFATE AND ANIONIC POLYELECTROLYTES AS COAGULANT AIDS

Average Input Virus Concentration: 2.76×10^5/ml; pH range: 5.9 - 6.0

Aluminum Sulfate: 50 mg/l; Average Turbidity: 12.5 JTU

Anionic Polyelectrolyte	Concentration mg/l	Percent Removal	
		Bacteriophage MS2	Turbidity
	0.0	99.78	97.50
	0.5	99.70	96.58
Primafloc A-10	1.0	99.34	94.42
	2.5	98.75	88.30
	5.0	97.68	83.40
	0.0	99.61	97.20
	1.0	98.55	89.60
Coagulant Aid #243	2.0	97.53	90.40
	5.0	98.36	89.60
	10.0	95.22	88.00

the polyelectrolyte molecules, which resulted in subsequent infection of one host bacterium by more than one phage particle during phage assay. However, no attempt was made to reactivate or to free the phage particles from the polyelectrolyte. The anionic polyelectrolytes, Primafloc A-10 and Coagulant Aid #243, did not inactivate T4 and MS2 particles. This was due to the absence of cationic adsorption sites on the polyelectrolyte molecules.

It is seen from Table 17 that both the cationic polyelectrolytes used were quite effective as coagulant aids for bacteriophage T4 removal in the dosage range 0.5 to 1.0 mg/l. Turbidity removal was less efficient at higher dosages even though virus removal was higher. This was presumably due to virus inactivation by the polyelectrolyte per se. No improvement was noticed in the removal of bacteriophage MS2 (Table 18). Both the cationic polyelectrolytes were quite effective as prime coagulants (Tables 19 and 20). Primafloc C-7 and Catfloc in the dosage ranges 5.0 to 7.5 mg/l and 10.0 to 12.5 mg/l, respectively, gave the best results from the viewpoint of both virus and turbidity removal. Neither of the anionic polyelectrolytes used were effective as coagulant aids (Tables 21 and 22). This was presumably due to the absence of a sufficient concentration of calcium ions in the system which has been thought to be necessary for the action of anionic polyelectrolytes (Packham, 1967). This was also the reason for not using anionic polyelectrolytes as prime coagulants in this study.

D. Qualitative Description of Virus Removal by Chemical Coagulation and Flocculation

For purposes of attaining a better understanding of the removal of viruses from water by chemical coagulation and flocculation, it seems appropriate to present a qualitative description of the process. On the basis of the

results obtained in this study and the information available in the literature, it is possible to visualize the entire process.

In a natural surface water, depending on the concentrations of cations like sodium and calcium, a certain fraction of the virus particles present remain reversibly adsorbed to the clay particles constituting turbidity due to the formation of a clay-cation-virus bridge (Carlson et al., 1968). The other fraction may be assumed to be free. Addition of a coagulant like aluminum sulfate to this water immediately results in the formation of certain hydrolyzed polymeric multivalent aluminum species depending on the pH of the water (Black and Chen, 1967). Interaction between aluminum species and viruses, other organic matter, and clay particles proceeds immediately. The interaction between aluminum and viruses and other organic matter is a very rapid one and presumably results in the formation of coordination complexes. On the other hand, the interaction between aluminum and the clay particles constituting turbidity results in adsorption of polynuclear aluminum hydrolysis species to the clay particles and consequent aggregation of the destabilized particles by interparticle bridging involving particle transport and chemical interaction (Stumm and O'Melia, 1968). Furthermore, precipitation of hydrated aluminum oxide species proceeds simultaneously, incorporating the complexed virus particles and the aggregating clay particles which then grow into "flocs" and ultimately settle down resulting in a clear supernatant. Presence of organic matter in the water can have considerable effect on the overall efficiency of the process by interfering with virus removal. This may occur because of the competitive action of the organic matter with the virus particles in the coagulation and flocculation reaction.

VI. SUMMARY AND CONCLUSIONS

It has been shown that removal of bacteriophages T4 and MS2 from water by chemical coagulation and flocculation with aluminum sulfate consists of a primary reaction step which involves interaction between aluminum and virus coat protein. The reaction was found to be instantaneous and proceeded according to a definite stoichiometry. The kinetics and the stoichiometry of the reaction were not affected by the pH, period and condition of storage of the virus particles, the quantity of available aluminum or the presence of bivalent cations like calcium and magnesium as studied in this investigation.

Aluminum adsorption data were found to fit Langmuir adsorption equation. Amounts of aluminum adsorbed by a single virus particle at pH values 5.0, 6.0, and 9.0 were calculated and found to be comparable. Considering the aqueous chemistry of aluminum, amino acid composition of the virus coat protein and the evidences of an aluminum-protein interaction as reported in the literature, it was concluded that the interaction between aluminum and virus possibly resulted in the formation of coordination complexes between aluminum and the carboxyl groups associated with the virus coat protein. The complexed viruses were not inactivated and active viruses could be recovered from the settled floc following their removal from water by coagulation and flocculation.

The process of chemical coagulation and flocculation was found quite effective in removing bacteriophages T4 and MS2 from water. The optimum coagulant dosages and pH values were 40 to 50 mg/l of aluminum sulfate at pH 5.24 for bacteriophage T4 and at pH 6.0 for bacteriophage MS2. The highest removals attained were 98.0 and 99.9 percent, respectively. Presence of bivalent cations like calcium and magnesium up to a concentration of 50 mg/l each did not interfere with the efficiency of the process. Organic matter like

albumins and that associated with wastewater lowered the removal efficiency considerably. Commercially available cationic polyelectrolytes were found effective both as coagulant aids and as prime coagulants.

Based on the findings of this investigation, the following conclusions may be drawn:

(i) Removal of viruses by chemical coagulation and flocculation with aluminum sulfate comprises of a primary instantaneous reaction step which results possibly in the formation of coordination complexes between aluminum and the carboxyl groups of the virus coat protein.

(ii) Virus particles are not inactivated as a result of this interaction between aluminum and the virus particles and remain active in the settled sludge following their removal from water by coagulation and flocculation with aluminum sulfate.

(iii) Chemical coagulation and flocculation is an effective process in removing viruses from water. Removals in the range 98.0 to 99.9 percent can be expected.

(iv) Presence of bivalent cations like calcium and magnesium up to a concentration of 50 mg/l each does not interfere with the efficiency of the process.

(v) The efficiency of virus removal is reduced when the raw water contains organic matter.

(vi) Intelligent use of commercially available cationic polyelectrolytes with or without hydrolyzed metal ions may markedly increase the efficiency of the coagulation and flocculation process.

It seems appropriate to note the recent observations made by Stumm and O'Melia (1968):

> "It is important to reemphasize that coagulation phenomena in natural systems are quite specific. This specificity arises

from the fact that colloid stability is affected by colloid-solvent, coagulant-solvent, and colloid-coagulant interactions..... Overemphasis on electrostatic phenomena in studies of coagulation in natural systems can produce results that are inefficient, uneconomical, or both."

VII. ENGINEERING SIGNIFICANCE

The most significant result of this study is that a more complete understanding of the removal of viruses from water by chemical coagulation and flocculation has been attained. This is very pertinent in view of the more stringent water quality standards which are foreseen in the near future. Considering the rapid population growth and accompanying urbanization, there is going to be a greater demand for water for public consumption and other uses which, in turn, will require more water reuse. Thus, the removal of viruses from water supplies becomes extremely important. A good understanding of the basic mechanisms involved in the removal of viruses from water by chemical coagulation and flocculation and the role of various other parameters affecting the process should aid in developining design standards for water treatment facilities. It will enable such standards to be developed on a sound, realistic and rational basis.

From the experimental results it is possible to extrapolate some generalizations which are of practical significance. The foremost among the generalizations is the interpretation of T4 and MS2 removal data in terms of viruses which may be more significant in water supplies, viz., human enteric viruses. Confirmation of T4 removal data by MS2 which is very similar to picornaviruses (enteroviruses of man and other animals) in shape, size and the nucleic acid contained, shows that the process of chemical coagulation and flocculation may be quite effective in removing enteroviruses from water. From the results of jar tests using polyelectrolytes it is apparent that intelligent use of commercially available cationic polyelectrolytes with or without hydrolyzed metal ions may markedly increase the efficiency of the process. However, from economic

considerations, the use of the cationic polyelectrolytes as coagulant aids with hydrolyzed metal ions seem more favorable. Based on today's market, polyelectrolytes are rather expensive.

From the results of virus inactivation studies and virus recovery from settled floc it is possible to extrapolate some useful information. The observation that viruses are not inactivated as a result of the complex formation and remain viable in the settled sludge immediately leads to the potential hazard for ground water contamination during land disposal of sludges from water treatment plants treating water contaminated with pathogenic viruses. However, more information should be gained in this area such as the fate including the survival of the viable viruses in sludge during land disposal before any definite conclusion can be reached.

On the basis of the findings of Chang, Isaac and Baine (1953) that approximately 20 min would be required for the completion of the first-stage reaction (aluminum-virus complex formation) it may be speculated that the incremental addition of the coagulant may be advantageous in optimizing the process for virus removal. In such a process the procedure might be to first add aluminum sulfate to the raw water in an amount equivalent to or less than its solubility, taking into account the pH of the system; second, provide sufficient contact time (20 min) for the formation of the aluminum-virus complex; and, third, add sufficient aluminum sulfate to bring the total amount added up to the predetermined optimum dosage for coagulation and flocculation. Following the second addition of coagulant, the usual period of flocculation and sedimentation would be included. However, the findings of the current study that the interaction between the aluminum and the virus is instantaneous immediately rules out such a speculation.

The observation that the presence of organic matter interferes with virus removal by coagulation and flocculation leads to another generalization. For virus removal, the process may be more reliable as practiced at a water treatment plant than at a wastewater renovation plant because of the presence of higher concentration of organic matter in a wastewater effluent.

The findings of this study indicate that virus removal by coagulation and flocculation parallel turbidity removal. Robeck, Clarke and Dostal (1962) observed that effective coagulation and flocculation was an essential prerequisite for effective virus removal by rapid sand filtration. They also observed that any breakthrough in turbidity through the filter was accompanied by a breakthrough in virus. Consequently, it may be suggested that in a water treatment plant care should be taken to produce a high quality effluent in terms of turbidity. Any breakthrough in turbidity should serve as a warning to the operator. This is particularly important during heavy pollution of the raw water and may become more critical when marginal chlorination is practiced.

Finally, the results of this study suggest that the process of chemical coagulation and flocculation as it is practiced today can be quite effective in removing pathogenic viruses from water if proper care is taken to control pH and other parameters which affect the process. It should be noted that pH was not found to be an important variable with respect to the formation of the aluminum-virus complex; however, such is not the case when both coagulation and flocculation are considered.

VIII. SUGGESTIONS FOR FUTURE WORK

On the basis of the results of the current study it is felt that further investigations should be pursued in the following areas.

(i) Studies should be made to evaluate the efficiency of the chemical coagulation and flocculation process in removing viruses which may be significant in water supplies, viz., polioviruses. Initial virus concentrations to be used in these studies should be comparable to the expected density of enteric viruses in a polluted surface water. This would require the use of concentration techniques for virus detection following their removal from water by chemical coagulation and flocculation.

(ii) Virus removal efficiency of the process of chemical coagulation and flocculation should be evaluated when iron salts are used as coagulant.

(iii) More studies should be made to find the effect of pH on virus removal by chemical coagulation and flocculation using commercial polyelectrolytes as prime coagulants and coagulant aids.

(iv) Investigations should be undertaken to find the fate of viable viruses in water treatment plant sludge during land disposal in order to assess its potential as a source of ground water contamination, if any.

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